MANGANESE SALTS VOL I #68

K19

Manganese Salts-Volume 1 #68 11/9/73

VOLUME 1

GRAS MONOGRAPH SERIES

MANGANESE SALTS

prepared for
THE FOOD AND DRUG ADMINISTRATION
DEPARTMENT OF HEALTH, EDUCATION
AND WELFARE

NOVEMBER 9, 1973

This publication was prepared under Contract Number FDA 72-100
with the Public Health Service, Food and Drug Administration,
Department of Health, Education, and Welfare

prepared by

Tracor Jitco, Inc.

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Summary

Description and Specifications

1. Manganese chloride: Manganese chloride occurs as pink, cubic crystals when anhydrous, and large, irregular translucent crystals when it is in the tetrahydrate form. Manganese chloride has the formula $MnCl_2$ and molecular weight of 125.84. It is soluble in water and alcohol, and is insoluble in ether and ammonia. The Food Chemicals Codex (040) specifies that an assay of food grade manganese chloride show not less than 98.0% and not more than 102.0% $MnCl_2 \cdot 4H_2O$.

2. Manganese citrate: Manganese citrate is a white or reddish white powder in which the ratio of citric acid to manganese may be 1:1, 2:3, or an indeterminate proportion. Characteristic of manganese citrate as its insolubility in water, though it will dissolve in dilute acids and sodium citrate solutions.

3. Manganese citrate (soluble): Another form of manganese citrate which is referred to as soluble manganese citrate is an undefined complex of the manganese and sodium salts of citric acid. It contains 14.6-15.8% Mn equivalent to 48-52% tribasic manganese citrate.

4. Manganese gluconate: Manganese gluconate is a slightly pink powder with the empirical formula $C_{12}H_{22}MnO_{14} \cdot 2H_2O$ (040), though other combining forms exist. It is very soluble in water but only slightly soluble in alcohol.

5. Manganese orthophosphate: Manganese orthophosphate is a compound of molecular formula $Mn_3(PO_4)_2$, occuring also in the trihydrate and heptahydrate forms. The trihydrate form is the mineral reddingite.

6. Manganese phosphate (dibasic): Dibasic manganese phosphate is a pink powder (or red rhomboidal crystals) with the molecular formula $MnHPO_4 \cdot 3H_2O$ and molecular weight 204.97. It is insoluble in alcohol, slightly soluble in water, and soluble in dilute acids.

7. Manganese oxide: Manganese oxide of molecular formula MnO_2 and molecular weight 86.94 occurs naturally as the mineral pyrolusite which is a black powder (or steel-grey when in lumps). At 535°C it becomes the monoxide, MnO. It is insoluble in water, but it will dissolve in dilute nitric and sulfuric acid when hydrogen peroxide or oxalic acid are present. Manganese dioxide is a strong oxidizer and should not be placed in contact with organic matter.

8. Manganese sulfate: Manganese sulfate is an odorless pale pink granular mass with the molecular formula $Mn(SO_4 \cdot H_2O$ and a molecular weight of 169.01. It becomes anhydrous between 400-500°C, melts at 700°C and decomposes at 850°C. It is freely soluble in water, but insoluble in alcohol. The Food Chemicals Codex (040) specifies that an assay of food grade manganese sulfate show not less than 98.0% and not more than 102.0% $Mn(SO_4) \cdot H_2O$, with loss on heating (400-500°C) between 10 and 13%.

Acute Toxicity

Schroeder et al. (222) noted that manganese was the least toxic trace metal to fish. Vertebrates have been shown to be able to tolerate large concentrations of aqueous manganous ion, Mn^{2+} (See Acute Toxicity Table).

Handovsky et al. (092), found the LD_{100} for subcutaneous administration of manganese chloride ($MnCl_2$) or manganese citrate [$Mn_3(C_6H_5O_7)_2$] to mice, rabbits and guinea pigs to be 50 mg/kg BW, death occurring at a maximum of 12 hours following administration.

Ceasar and Schnieden (034) determined the LD_{50} (one-hour) for intraperitoneal injection of manganese sulfate ($MnSO_4$) to be 534 mg/kg BW in mice.

Mackiewicz (152) subcutaneously injected $MnCl_2$ into rats and found the LD_{50} to be 50 mg/kg BW and the LD_{100} to be 60 mg/kg BW.

Kobert (cited by Von Oettingen, 269) found the LD_{100} for subcutaneous injection of manganese oxide (MnO) to guinea pigs to be 28-30 mg/kg BW. He also found the subcutaneous LD_{100} dosage of MnO to be 12-13 mg/kg BW for rabbits.

Cervinka (035) found the average lethal dose of $MnCl_2$ mixed with an unstated amount of sodium thiosulfate and intravenously injected into rabbits to be 18 mg Mn/kg BW. He also found the average lethal dose of the same mixture intravenously injected into dogs to be 56 mg Mn/Kg BW.

Sabbatini (219) found the LD_{100} for intravenous injection into rabbits to be:

(1) for $MnCl_2$, 21.4 mg/kg BW

(2) for $MnCO_3$, 21.3 mg Mn/kg BW

(3) for $Mn_3(PO_4)_2$ (colloidal), 23 mg Mn/kg BW

Kobert (cited in Von Oettingen, 269) found the LD_{100} (24 hours) for subcutaneous injection of MnO to dogs to be 13-14 mg/kg BW and the LD_{100} (two days) to be 6-8 mg/kg BW.

Short-Term Toxicity

Tal and Guggenheim (253) maintained mice on meat diets supplemented with $MnSO_4$ (from 2.5 to 25.0 mg/kg of meat) for from two to six weeks. They found that doses of from 10.0 to 25.0 mg/kg of meat administered over a prolonged period were toxic and induced defective bone calcification. Findlay (071) found that when rats were subcutaneously injected with 3 mg doses of $MnCl_2$ that varying degrees of liver fibrosis developed. When the manganese salt was added as a dietary supplement (0.3-0.4 g/day), all the animals died within six to ten weeks and showed some degree of liver cirrhosis.

Moinuddin and Lee (175) supplemented the diets of rats with $MnSO_4 \cdot H_2O$ in varying amounts from very low (0.88 m mole /kg of feed) to very high (138 m mole/kg of feed) for about a month. Effects were found only at the highest dose level, with the most noteworthy being depigmentation of the labial surfaces of the incisor teeth, reduced hemoglobin and a higher erythrocyte count, and reduced serum inorganic phosphorus.

Levina and Minkina (145) found that when rats were subcutaneously injected with 100 mg/kg BW of either MnO_2 or MnO for a month, the animals showed toxic effects during the course of the experiment. Autopsy disclosed increased size and weight of the adrenals. In general there was a marked loss of the ketosteroids and decrease in ascorbic acid content.

Chandra and Srivastava (036) produced brain lesions in rats with intraperitoneal injections of 8 mg/kg for 120 days. They concluded that the severity of lesions was directly related to the manganese concentration in the brain.

Voigt and Saldeen (266) produced severe liver degeneration as well as necroses of the epithelial cells in the kidney and small necroses in the myocardium of hamsters subcutaneously injected with 4.5 mg/100 g BW $MnSO_4$ for 10 days.

Findlay (071) produced liver fibrosis in guinea pigs, similar to those produced in rats, by subcutaneous injections of 3 mg $MnCl_2$ in aqueous solution. Findlay (071) also produced liver cirrhosis in rabbits (as with rats and guinea pigs) by either single subcutaneous injections of 10 to 60 mg aqueous $MnCl_2$ or repeated subcutaneous injections of 3 or 5 mg. The author suggested that this toxic effect of $MnCl_2$ may be related to its excretion, which is largely via the bile.

Matrone et al. (158) found that hemoglobin regeneration over a 6-week period in anemic rabbits was slower when supplementary manganese (2000 ppm $MnSO_4 \cdot H_2O$) was added to their diets.

Oettel (189) produced liver sclerosis in 10 weeks in large dogs by intramuscular injections with 20 mg/10 kg BW every two days.

Hartman et al. (096) found that feeding manganese supplemented diets (as $MnSO_4 \cdot H_2O$) to iron-depleted lambs decreased their hemoglobin and serum iron concentration even at supplemental levels as low as 45 ppm. Feeding levels of 1000 ppm or 2000 ppm Mn as $MnSO_4 \cdot H_2O$ retarded hemoglobin regeneration. The lambs on the Mn supplemented diets also had lowered serum iron concentrations. The authors suggested that Mn interferes with iron absorption.

Matrone et al. (158) found that excessive manganese (as $MnSO_4$) added to the diets of anemic baby pigs retarded hemoglobin regeneration. The minimum level of dietary Mn found to interfere with hemoglobin formation was between 50 and 125 ppm.

Pentschew et al. (200) found that monkeys injected intramuscularly with 2000 mg and 3500 mg doses of MnO_2 suspended in olive oil developed neurological disturbances. Autopsy showed alterations in the brain identical with those seen in human cases of manganese encephalopathy. The salient feature in both man and primates appears to the authors to be the severe selective damage to the subthalamic nucleus and pallidum.

Kawamura et al. (121) reported conclusive proof of poisoning in humans due to ingestion of dissolved manganese. The symptoms, affecting 16 people, resembled those associated with a motor disturbance of the extrapyramidal region, beginning with lethargy and edema. The poisoning was traced to ingestion of well water excessively high in manganese (ca. 14 mg/l).

Long-Term Toxicity

Becker and McCollum (012) fed a small number of rats a basal diet supplemented with 3.6% $MnCl_2 \cdot 4H_2O$ for 730 days. They found the sexual function of the males on a Mn supplemented diet was retained longer than was the case in rats on an unsupplemented diet.

Special Studies

Demerec and Hanson (056) found that the mutagenic effectiveness of $MnCl_2$ in the B/r strain of Escherichia coli could be made to vary by a factor as high as 2.5×10^4 by changing certain physiological conditions of the treated organism. The results of their experiments indicated that, while highly sensitive to outside agents, the mutagenic effect of $MnCl_2$ was general.

Steinman et al. (249) found that when $MnCl_2$ was applied to bacterial colonies for a few hours at 0.04% concentration, its mutagenic properties were appreciably potentiated by some but not by other mutagens.

Elwood (064) found that injected $MnCl_2$ (400 mg/kg BW, single injection), produced amelogenic disturbances and enamel defects in the incisors of all the rats treated. He also produced hypoplastic enamel defects in the incisors of guinea pigs with subcutaneously injected $MnCl_2$ (150-490 mg/kg BW). No defects were produced in hamsters however.

Walbum and Schmidt (275) found that injected $MnCl_2$ was effective in the formation of amboreceptors in rabbits. Gorlitzer (084) reported the successful treatment of 17 cases of "rheumatic" endocarditis and one case of acute tonsillitis with intravenous injections of a 0.02 molar solution of $MnCl_2$.

Mehrotra et al. (164) produced a statistically significant decrease in blood sugar levels in 15 diabetics with oral administration of varying doses of $MnCl_2$ with 50 g glucose. Artamonova (006) obtained good or satisfactory results in the treatment of 22 cases of non-specific infectious polyarthritis with daily intramuscular injections of a 1% aqueous $MnCl_2$ solution (0.1 ml to 2.0 ml).

Breakdown

There is no specific information in the literature concerning the breakdown of manganese compounds in the body. However, Von Oettingen (269) has pointed out that the difference in the degree of solubility of the various manganese compounds is reflected in the level of manganese found in the blood stream following administration.

Absorption and Distribution

Cikrt (038) used ^{52}Mn (as the chloride) to study the uptake in the duodenal and ileal segments of the small intestine. He concluded that there is an active transport of Mn through the intestinal wall *in vitro*.

Britton and Cotzias (030) used injected $^{54}MnCl_2$ to observe the effect of stable manganese intake (as $MnSO_4$ dietary supplement) on the distribution rate in mice. They found that manganese turnover in the tissues is dependent on the supply.

Lemos (040) studied the distribution of manganese following administration of different manganese compounds by various routes in rabbits and guinea pigs. The most unexpected finding was the high levels of Mn found in the suprarenals, bone marrow, testicles and spleen.

Cotzias *et al.* (047) compared the rate of loss of injected ^{54}Mn from the whole body and from an area representing the liver, between a normal population of "healthy" manganese miners and patients suffering from chronic manganese poisoning. The authors concluded from their experimental findings that elevated tissue concentrations are related to exposure but are not necessary for the presence of neurological symptoms of chronic manganese poisoning.

Metabolism and Excretion

Cotzias and Greenough (046) concluded from their investigations of the manganese pathway through the mouse's body that there is a specific intracellular manganese pathway. They also demonstrated that manganese performs

specific tasks which cannot be taken over by other metals, by showing that manganese could not be replaced by other metals (ferrous ion and chromous ion).

Hughes et al. (108) studied the effects of administering ACTH or cortisol and the effect of adrenalectomy on the excretion and tissue distribution of manganese in mice, in order to ascertain whether there was endocrine regulations of manganese metabolism. They concluded that manganese metabolism was under a regulatory control whose precise nature was not yet understood.

Papavasiliou et al. (194) examined the effect of obstruction of the rectum and bile flow on the distribution and excretion of manganese in rats. It was found that under ordinary conditions, bile formation is the main regulatory route but excretion by auxiliary routes occurs when there is overloading or blockage of the biliary route.

Bertinchamps et al. (015) identified the auxiliary routes of Mn excretion as the duodenum and jejunum.

Borg and Cotzias (025) analyzed the blood clearance and liver uptake of radiomanganese in humans. They found that blood manganese and liver mitochondrial manganese rapidly enter equilibrium.

Effect on Enzymes and other Parameters

Shimatami (227) found that $MnCl_2$ inhibited both the dephosphorylating (hydrolyzing) and phosphorylating action of the mucosa enzyme, phosphatase.

Scrutton et al. (223) identified pyruvate carboxylase as a manganese metalloprotein with between 2.5 to 4.3 moles Mn/mole enzyme.

Mildvan et al. (171) concluded from their investigations that the tightly bound Mn in pyruvate transcarboxylase functions in the transcarboxylation part of this enzyme.

Amdur et al. (002) found that manganese (as $MnCl_2$) prevents the deposition of excess fats in rat livers. As further evidence for the lipotropic action of Mn, it was found that supplementary dietary Mn also caused a significant reduction in the percentage of fat in fresh bone.

Gubler et al. (090) found that administration of large amounts of manganese and copper simultaneously to rats (4% $MnDl_2$ and 0.1% copper sulfate in basal diet) increased the copper concentration markedly in the total body as well as in the plasma, liver, kidneys and brain. The authors suggested the possibility that manganese either complexes with copper thus making it unavailable, or that is somehow blocks the action of copper-containing enzymes.

Mosendz and Silakova (178) found the $MnCl_2$ increased the tissue ammonia content in rats while at the same time the processes neutralizing its toxicity were also intensified, i.e., the amount of both glutamine and amide nitrogen increased.

Everson and Schrader (067) showed that manganese is involved in glucose utilization. Manganese supplementation completely reversed reduced glucose utilization in Mn-deficient guinea pigs.

Maksimov and Laskavaya (153) found that $MnCl_2$ administration may alter the glucocorticoid function of the adrenal cortex in guinea pigs and that at the effective $MnCl_2$ dosage (i.e., at the dosage where the content of the 17-hydroxycorticosteroids in plasma are affected) the amount of ascorbic acid in the adrenals is also altered.

Rubenstein et al. (218) reported a hypoglycemic effect on a diabetic patient administered manganese (either in the plant, lucerne or as $MnCl_2$) as evidenced by modification of the glucose tolerance curve.

Drug Interaction

Hughes et al. (108) found that cortisol acetate affected manganese metabolism in mice. They suggested the possibility that since glucocorticoid hormones are administered to man in large amounts and for long periods of time, they should be investigated to determine whether they produce syndromes in man related to experimental manganese deficiency.

Comens (039) studied the effect of the manganous ion (as manganese citrate) on "hydralazine disease" in cockerels, dogs and humans. He found the Mn^{2+} (5 mg per day) prevented the appearance of symptoms in the experimental animals and improved the condition in patients. He concluded that hydralazine may act by binding Mn^{2+} and thus blocking enzyme systems.

Shugaylo and Gude (235) found that the combined administration of 0.1 to 0.05% $MnCl_2$ solution with vitamins C and B_1 alleviated the symptoms of infectious hepatitis more quickly than vitamin therapy alone.

Consumer Exposure

Manganese chloride, manganese gluconate, and manganese sulfate are listed in the Food Chemicals Codex (040) as nutrients and dietary supplements. The 1972 NAS/NRC report (073) listed the average amount of manganese chloride added to infant formulas as 313 ppm, and that for manganese sulfate as 27 ppm. In 1962, the United States Public Health Service set the maximum level of manganese in drinking water at 0.05 mg/liter (004). Estimates of manganese consumption from the trace amounts naturally present in foods range from 2.3 to 7.5 mg Mn/day (262).

CHEMICAL INFORMATION

Manganese Chloride

I. **Nomenclature**

A. Common names: Scacchite ($MnCl_2$), naturally occurring mineral

B. Chemical names: Manganese chloride; manganous chloride; manganese chloride, tetrahydrate; manganese dichloride

C. Trade names: none

D. Chemical Abstracts Service Unique Registry Number:

7773015 (anhydrous)

II. **Empirical formula**: Cl_2Mn (anhydrous)

$Cl_2Mn \cdot 4\ H_2O$ (tetrahydrate)

III. **Structural formula**: $MnCl_2$ (anhydrous)

$MnCl_2 \cdot 4\ H_2O$ (tetrahydrate)

IV. **Molecular weight**: 125.84 (anhydrous)

197.91 (tetrahydrate)

V. **Specifications**:

A. The Food Chemicals Codex, Second Edition, presents the following specifications for food grade manganese chloride (tetrahydrate) (040):

"Description
Large, irregular, pink, translucent crystals. It is freely soluble in water at room temperature and very soluble in hot water.

Identification
A 1 in 20 solution gives positive tests for Manganese, page 927, and for Chloride, page 926.

Specifications
Assay. Not less than 98.0 percent and not more than the equivalent of 102.0 percent of $MnCl_2 \cdot 4H_2O$.
pH of a 5 percent solution. Between 4.0 and 6.0.
Limits of Impurities
Arsenic (as As). Not more than 3 parts per million (0.0003

percent).
Heavy metals (as Pb). Not more than 10 parts per million (0.001 percent).
Insoluble matter. Not more than 50 parts per million (0.005 percent).
Iron. Not more than 5 parts per million (0.0005 percent).
Substances not precipitated by sulfide. Not more than 0.2 percent, after ignition.
Sulfate. Not more than 50 parts per million (0.005 percent).

Tests

Assay. Tranfer about 4 grams, accurately weighed, into a 250-ml. volumetric flask, dissolve in water, dilute to volume with water, and mix. Transfer 25.0 ml. of this solution into a 400-ml. beaker, and add 10 ml. of a 1 in 10 solution of hydroxylamine hydrochloride, 25 ml. of 0.05 M disodium ethylenediaminetetraacetate measured from a buret, 25 ml. of ammonia-ammonium chloride buffer T.S., and 5 drops eriochrome black T.S. Heat the solution to between 55° and 65°, and titrate from the buret to a blue end-point. Each ml. of 0.05 M disodium ethylenediaminetetraacetate is equivalent to 9.896 mg. of $MnCl_2 \cdot 4H_2O$.

pH of a 5 percent solution. Determine by the Potentiometric Method, page 941.

Arsenic. A solution of 1 gram in 35 ml. of water meets the requirements of the Arsenic Test, page 865.

Heavy metals. A solution of 2 grams in 25 ml. of water meets the requirements of the Heavy Metals Test, page 920, using 20 mcg. of lead ion (Pb) in the control (Solution A).

Insoluble matter. Dissolve about 20 grams, accurately weighed, in 200 ml. of water, and allow to stand on a steam bath for 1 hour. Filter through a tared sintered glass crucible, wash thoroughly with hot water, dry at 105° for 1 hour, cool, and weigh.

Iron. Dissolve 2.0 grams in 20 ml. of water, add 1 ml. of hydrochloric acid, and dilute to 50 ml. with water. Add about 40 mg. of ammonium persulfate crystals and 3 ml. of ammonium thiocyanate T.S. Any red or pink color does not exceed that produced by 1.0 ml. of Iron Standard Solution (10 mcg. Fe) in an equal volume of a solution containing the quantities of the reagents used in the test.

Substances not precipitated by sulfide. Dissolve 2.0 grams in about 90 ml. of water, add 4 ml. of ammonium hydroxide, heat to 80°, and pass hydrogen sulfide through the solution to completely precipitate the manganese. Dilute to 100 ml., mix, and allow the precipitate to settle. Decant the supernatant liquid through a filter, and evaporate 50 ml. of the filtrate to dryness in a tared dish. Add 0.5 ml. of sulfuric acid, ignite to constant weight, cool, and weigh.

Sulfate. Dissolve 10.0 grams in 100 ml. of water, add 1 ml. of diluted hydrochloric acid T.S., mix, and filter. Heat to boiling, then add 10 ml. of barium chloride T.S., and allow to stand overnight. Filter out any precipitate in a tared crucible, wash, ignite gently, cool, and weigh. The weight of the ignited precipitate should not be more than 1.2 mg. greater than the weight

obtained in a complete blank test.
Packaging and storage. Store in well-closed containers.

Functional use in foods. Nutrient; dietary supplement."

VI. Description

A. General characteristics: Manganese chloride occurs as pink, cubic crystals which are deliquescent. The tetrahydrate crystals are large, translucent, irregular and pink to rose or reddish in color.

B. Physical properties: Manganese chloride has a density of 2.977 (at 25°C relative to water at 4°C). It has a melting point of 650°C and it boils at 1190°C. 72.3 g are soluble in 100 ml of water at 25°C and 123.8 g dissolve in 100 ml of water at 100°C. It is also soluble in alcohol and is insoluble in ether and ammonia.

The tetrahydrate has a density of 2.01. It has a melting point of 58°C. At 106°C it loses one molecule of water of hydration and it becomes anhydrous at 198°C. 151 g dissolve in 100 ml of water at 8°C and 656 g are soluble in 100 ml of water at 100°C. It is also soluble in alcohol and is insoluble in ether. The pH of a 0.2 molar aqueous solution is 5.5.

C. Stability in containers: No information available.

I. Nomenclature

A. Common names: none

B. Chemical names: Manganese citrate; manganese (II) citrate; citric acid, manganese(2+) salt (2:3); citric acid, manganese(2+) salt (1:1)

C. Trade names: none

D. Chemical Abstracts Service Unique Registry Number:

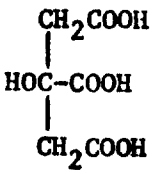
10377410 ($O_7C_6H_8$ · 3/2 Mn)

5968887 ($O_7C_6H_8$ · Mn)

10024665 ($O_7C_6H_8$ · x Mn)

II. Empirical formula: $C_{12}H_{16}O_{14}Mn_3$

III. Structural formula:



· 3/2 Mn

IV. Molecular weight: 543.02

V. Specifications: not available

VI. Description:

A. General characteristics: Manganese citrates (both $O_7C_6H_8$ · 3/2 Mn and $O_7C_6H_8$ · Mn) occur as white or reddish-white powders.

B. Physical properties: Manganese citrate is only very slightly soluble in water, but is soluble in dilute acids and sodium citrate solutions.

C. Stability in containers: No information available.

Manganese Citrate, Soluble

I. Nomenclature

A. Common names: Manganese citrate, soluble

B. Chemical names: Manganese sodium citrate

C. Trade names: none

D. Chemical Abstracts Service Unique Registry Number: not available

II. Empirical formula: Soluble manganese citrate is not a defined structural complex. It contains 14.6-15.8% Mn equivalent to 48-52% tribasic manganese citrate.

III. Structural formula: not defined

IV. Molecular weight: not defined

V. Specifications: not available

VI. Description:

A. General characteristics: Soluble manganese citrate occurs as a pinkish-white powder.

B. Physical properties: Soluble manganese citrate is soluble in about 4 parts water (slightly more so in boiling water) and is almost insoluble in alcohol.

C. Stability in containers: No information available.

Manganese Gluconate

I. Nomenclature

A. Common names: none

B. Chemical names: Manganese gluconate

C. Trade names: none

D. Chemical Abstracts Service Unique Registry Number:

6485398 ($O_7C_6H_{12}$ · 1/2 Mn)

12261904 ($O_{14}Mn\ C_{12}H_{22}$)

12261915 ($O_{14}Mn\ C_{12}H_{22}$ · OH_2)

II. Empirical formula: $C_{12}H_{22}MnO_{14}$ · $2H_2O$ (Food Chemicals Codex, 040)

III. Structural formula:

$$[CH_2OH(CHOH)_4COO]_2Mn \cdot 2H_2O$$

IV. Molecular weight: 481.27 (based on empirical formula above)

V. Specifications:

A. The Food Chemicals Codex, Second Edition, presents the following specifications for food grade manganese gluconate (040):

"Description

A slightly pink colored powder. It is very soluble in hot water and is very slightly soluble in alcohol.

Identification

A. A 1 in 20 solution gives positive tests for Manganese, page 927.

B. It meets the requirements of Identification Test B under Copper Gluconate, page 219.

Specifications

Assay. Not less than 98.0 percent of $C_{12}H_{22}MnO_{14}$ · $2H_2O$.

Limits of Impurities

Arsenic (as As). Not more than 3 parts per million (0.0003 percent).

Heavy metals (as Pb). Not more than 40 parts per million (0.004 percent).

Lead. Not more than 10 parts per million (0.001 percent).
Reducing substances. Not more than 0.5 percent.

Tests

Assay. Dissolve about 600 mg., accurately weighed, in 50 ml. of water in a 250-ml. porcelain casserole, add 1 gram of hydroxylamine hydrochloride, 10 ml. of ammonia-ammonium chloride buffer T.S., and 5 drops of eriochrome black T.S., and titrate with 0.05 M disodium ethylenediaminetetraacetate to a deep blue color. Each ml. of 0.05 M disodium ethylenediaminetetraacetate is equivalent to 24.06 mg. of $C_{12}H_{22}MnO_{14} \cdot 2H_2O$.

Arsenic. A solution of 1 gram in 35 ml. of water meets the requirements of the Arsenic Test, page 865.

Heavy metals. A solution of 500 mg. in 25 ml. of water meets the requirements of the Heavy Metals Test, page 920, using 20 mcg. of lead ion (Pb) in the control (Solution A).

Lead. A solution of 1 gram in 25 ml. of water meets the requirements of the Lead Limit Test, page 929, using 10 mcg. of lead ion (Pb) in the control.

Reducing substances. Determine as directed in the test for Reducing Substances under Copper Gluconate, page 219.

Packaging and storage. Store in well-closed containers.

Functional use in foods. Nutrient; dietary supplement."

VI. Description:

A. General characteristics: Manganese gluconate occurs as a slightly pink colored powder.

B. Physical properties: Manganese gluconate is very soluble in hot water and is very slightly soluble in alcohol.

C. Stability in containers: No information available.

Manganese Orthophosphate

I. Nomenclature

A. Common names: Reddingite ($Mn_3(PO_4)_2 \cdot 3\ H_2O$), naturally occurring mineral.

B. Chemical names: Manganese orthophosphate; manganese (II) orthophosphate; phosphoric acid, manganese(2+) salt (2:3)

C. Trade names: none

D. Chemical Abstracts Service Unique Registry Number:

10236392 (heptahydrate)

II. Empirical formula: $O_8P_2Mn_3$ (also as tri and heptahydrate)

III. Structural formula: $Mn_3(PO_4)_2$ ($\cdot\ 3\ H_2O$ or $\cdot\ 7\ H_2O$)

IV. Molecular weight: 354.79 (anhydrous)

408.80 (trihydrate)

480.88 (heptahydrate)

V. Specifications: Not available.

VI. Description:

A. General characteristics: The trihydrated manganese orthophosphate occurs as rose or yellowish white rhomboidal crystals.

B. Physical properties: The specific gravity of manganese orthophosphate, trihydrate is 3.102.

C. Stability in containers: No information available.

Manganese Dioxide

I. Nomenclature

A. Common names: Pyrolusite (naturally occurring mineral), black manganese oxide, bog manganese, cement black, C.I. 77728, C.I. Pigment Black 14, C.I. Pigment Brown 9, manganese binoxide, manganese black, manganese brown, manganese superoxide, pyrolusite brown, Wad

B. Chemical names: Manganese dioxide

C. Trade names: none

D. Chemical Abstracts Service Unique Registry Number:

1313139

II. Empirical formula: MnO_2

III. Structural formula: MnO_2

IV. Molecular weight: 86.94

V. Specifications: Not available

VI. Description:

A. General characteristics. Manganese dioxide occurs naturally as the mineral pyrolusite, which is steel-grey when in lumps or black when powdered. When prepared artificially (by precipitation) it is a fine, brownish-black powder.

B. Physical properties. Manganese dioxide has a specific gravity of 5.026. The molecule loses one oxygen atom at 535°C. It is insoluble in water, nitric acid and cold sulfuric acid. It does dissolve in dilute nitric and sulfuric acid in the presence of hydrogen peroxide or oxalic acid. Manganese dioxide dissolves slowly in dilute hydrochloric acid with the evolution of Cl.

C. Stability in containers. Manganese dioxide is a strong oxidizer and should not be exposed to heat or placed in contact with organic matter or other easily oxidizable substances.

Manganese Phosphate, Dibasic

I. Nomenclature

A. Common names: none

B. Chemical names: Manganese phosphate, dibasic; manganese (II) orthophosphate, mono-H; phosphoric acid, manganese (2+) salt (1:1), trihydrate

C. Trade names: none

D. Chemical Abstracts Service Unique Registry Number:

7782765

II. Empirical formula: $HMnO_4P \cdot 3\ H_2O$

III. Structural formula: $MnHOP_4 \cdot 3\ H_2O$

IV. Molecular weight: 204.97

V. Specifications: Not available

VI. Description:

A. General characteristics: Dibasic manganese phosphate occurs as a pink powder or as red rhomboidal crystals.

B. Physical properties: Dibasic manganese phosphate has an index of refraction of 1.656. It is very slightly soluble in water, soluble in dilute acids and is insoluble in alcohol.

C. Stability in containers: No information available.

I. Nomenclature

A. Common names: none

B. Chemical names: Manganese sulfate; manganese sulfate, monohydrate; manganese (II) sulfate, monohydrate; sulfuric acid, manganese(2+) salt (1:1), monohydrate

C. Trade names: none

D. Chemical Abstracts Service Unique Registry Number:

10034965

II. Empirical formula: $MnO_4S \cdot 3\ H_2O$

III. Structural formula: $Mn(SO_4) \cdot 3\ H_2O$

IV. Molecular weight: 169.01

V. Specifications:

A. The Food Chemicals Codex, Second Edition, presents the following specifications for food grade manganese sulfate (040):

"Description
A pale pink, granular, odorless powder. It is freely soluble in water and is insoluble in alcohol.

Identification
A 1 in 10 solution gives positive tests for Manganese, page 927, and Sulfate, page 928.

Specifications
Assay. Not less than 98.0 percent and not more than the equivalent of 102.0 percent of $MnSO_4 \cdot H_2O$.
Loss on heating. Between 10 and 13 percent.
Limits of Impurities
Arsenic (as As). Not more than 3 parts per million (0.0003 percent).
Heavy metals (as Pb). Not more than 40 parts per million (0.004 percent).
Lead. Not more than 10 parts per million (0.001 percent).

Tests

Assay. Transfer about 4 grams, accurately weighed, into a 250-ml. volumetric flask, dissolve in water, dilute to volume with water, and mix. Transfer a 25.0-ml. portion of this solution into a 400-ml. beaker, and add 10 ml. of a 1 in 10 solution of hydroxylamine hydrochloride, 25 ml. of 0.05 M disodium ethylenediaminetetraacetate measured from a buret, 25 ml. of ammonia-ammonium chloride buffer T.S., and 5 drops of eriochrome black T.S. Heat the solution to between 55° and 65°, and titrate from the buret to a blue end-point. Each ml. of 0.05 M disodium ethylenediaminetetraacetate is equivalent to 8.450 mg. of $MnSO_4 \cdot H_2O$.

Loss on heating. Heat about 1 gram, accurately weighed, in a crucible tared in a stoppered weighing bottle, to constant weight at 400°-500°. Cool in a desiccator, transfer to the stoppered weighing bottle, and weigh.

Arsenic. A solution of 1 gram in 35 ml. of water meets the requirements of the Arsenic Test, page 865.

Heavy metals. A solution of 500 mg. in 25 ml. of water meets the requirements of the Heavy Metals Test, page 920, using 20 mcg. of lead ion (Pb) in the control (Solution A).

Lead. Dissolve 1 gram in 3 ml. of dilute nitric acid (1 in 2) and 10 ml. of water, and boil for 2 minutes. Cool, and dilute to 100 ml. with water. A 25-ml. portion of this solution meets the requirements of the Lead Limit Test, page 929, using 25 ml. of Ammonium Citrate Solution, 1 ml. of Potassium Cyanide Solution, 0.5 ml. of Hydroxylamine Hydrochloride Solution, and 2.5 mcg. of lead ion (Pb).

Packaging and storage. Store in well-closed containers.

Functional use in foods. Nutrient; dietary supplement."

VI. Description:

A. General characteristics: Manganese sulfate is a pale pink granular mass. The crystals are slightly efflorescent. It is odorless and has a strong alkaline taste.

B. Physical properties: Manganese sulfate becomes anhydrous at 400-500°C. The anhydrous form has a melting point of 700°C and it decomposes at 850°C. 52 g of anhydrous manganese sulfate is soluble in 100 ml of water at 5°C, while 70 g dissolves in 100 ml of 70°C water. The monohydrate is freely soluble in water and is insoluble in alcohol.

C. Stability in containers: No information available.

VII. Analytical Methods

Colorimetric methods

1. In 1917, Willard and Greathouse presented the periodate method for the colorimetric determination of manganese (278). Manganous salts are oxidized under acidic conditions with periodic acid (or its salts) to form the colored permanganate ion which is compared to standards. Although the method was presented for analyses of manganese in ores, a modification in which plant material is ashed and dissolved in hydrochloric acid was given official status in the 2nd Edition, 1925, of the Official Methods of Analysis of the Association of Official Agricultural Chemists (007) and remains the official method in the 10th edition, 1965 (008).

2. Davidson and Capen (052) modified the periodate method for manganese determination in plants by dissolving the ashed material in phosphoric or nitric acid to eliminate the troublesome reducing agent, hydrochloric acid, which was found to interfere in the assay.

3. Richards (214) found that the excess acid used by Willard and Greathouse tended to prevent full color development when dealing with smaller amounts of manganese, and therefore modified the procedure to call for less acidity in the oxidation of manganese when plant or animal materials are being assayed. Richards also removes excess chlorides by evaporation with concentrated sulfuric acid. (Ray (210) cautions against an excess of sulfuric acid which may form insoluble calcium sulfate, when calcium is present, and necessitate a filtration step.)

4. Mehling (162) studied the periodate method and found that the color produced is stable over a period of at least two months and that the color system follows Beer's Law. Interference due to anions could be overcome by excess periodate when these anions are present in moderate amounts. Cation interference was found with cupric, nickelous, cobaltous, chromic, uranyl, and ferric ions. Most of these cations would be removed in the course of the determination.

5. Formaldoxime has been used for the colorimetric determination of manganese. Bradfield (028) finds it necessary to use only one-fifth of the amount of material required by the periodate method for manganese determination.

Catalytic Methods

6. Because the periodate often requires 20-100 gm of material to determine the manganese content, other methods have been developed which utilize the permanganate ion as a catalyst for the oxidation of an organic compound. Wiese and Johnson (277) found that in an acid solution the permanganate ion will oxidize benzidine. The method is suitable for determining manganese in samples containing between 0.1 and 10 µg of manganese. Solutions must be kept acidic since alkaline solutions are found to fade rapidly. Sulfuric acid will precipitate the benzidine and must be avoided. Hydrochloric acid also interferes due to the chloride. Chlorides and other halogens must be removed by evaporation with nitric acid. Because excess oxidizing agents will impart a color to the benzidine, the manganese should be oxidized to permanganate by sodium bismuthate, since this compound forms insoluble bismuth oxide

in acid solutions and may be filtered off.

Durgakeri and Bellare (059) have modified the Wiese and Johnson method for manganese determinations in blood and serum.

7. Fore and Morton (077) used the permanganate ion to oxidize N,N-diethylaniline for determinations of microquantities of Mn. The rate of color development is directly proportional to the manganese concentration. The method is based on that of Kun (133). Fore and Morton found that a sample containing 1 ηg was the absolute minimum for determination and that in practice it should contain 5 ηg. The manganese concentration in the reaction mixture prepared for the determination could be measured to within ± 10% over a range of 0.4-4.0 ηg/ml. The pH is critical and must be controlled to within ± 0.1 unit. Iron interferes which excludes the use of this method in manganese determinations of blood or any material high in iron content.

8. Permanganate has also been used to catalyze the oxidation of 4,4'-tetramethyldiaminodiphenylmethane for determination of small amounts of manganese. The "tetrabase method", as it is referred to, has been found to be sensitive to 0.02 ppm Mn (043).

Gates and Ellis (082) have used 4,4'-tetramethyldiaminotriphenylmethane for manganese determinations and claim tests to show that the triphenyl compound is ten times more sensitive than the diphenyl.

Neutron activiation analysis

9. Undoubtedly the most precise and sensitive method for manganese determination is neutron activation analysis (174,193,027,048,069,149).

The great advantage of this technique is that following irradiation, one need not be concerned by manganese contamination. The major disadvantages are the equipment necessary for the procedure, the necessity of removing all sodium and chlorine (which are present in high amounts in all biological materials), and the need to deal systematically with a blank at least through the irradiation procedure and preferably through the entire analysis.

Meinke (165) compared the sensitivity of the neutron activation method with two different reactors to the "tetrabase method" (see above). He found the sensitivity of the neutron activation method to be 0.006 and 0.00003 µg/ml for the two reactors as compared to 0.001 µg/ml in the tetrabase procedure.

VIII. Occurrence

A. Plants

While manganese was observed to be a constant component of plant and animal tissue during the early twentieth century, it wasn't until 1923 that McHargue (161) presented the first evidence of a nutritional relationship. His work showed a plant requirement for manganese for normal growth and development. Accurate measurement of manganese occurrence in plants is limited by virtue of its usual presence in only minute or trace quantities. Reliability of the measurements is often questionable simply because manganese is a difficult element to quantify--particularly when the determination is on a biological substance. An additional factor which contributes to an apparent inconsistency in reported values of manganese occurrence in plant life

lies in the soil and season of growth. The variations were studied by Hopkins and Eisen (105) for manganese and several other mineral elements in nine of the most commonly eaten vegetables in this country.

Table 1 shows manganese content of some plant foodstuffs determined by Peterson and Skinner (201).

Table 1

Groups of Principal Foodstuffs Arranged in Descending Order of Manganese Content--Fresh Basis (Edible Portion)[a] (201)

Class of food	Number of samples	Manganese		
		Minimum	Maximum	Average
		mg. per kg.	mg. per kg.	mg. per kg.
Nuts	10	6.3	41.7	22.7
Cereals and their products	23	0.49	91.1	20.2
Dried legume seeds	4	10.7	27.7	20.0
Green leafy vegetables[b]	18	0.76	12.6	4.5
Dried fruits	7	1.5	6.7	3.3
Roots, tubers, and stalks	12	0.35	9.2	2.1
Fresh fruits, excluding blueberries	25	0.18	10.7	2.0
Fresh fruits, including blueberries	26	0.18	44.4	3.7
Non-leafy vegetables	5	0.82	2.4	1.5

[a]Determinations by Davidson and Capen modification (052) of the periodate method for manganese analysis.

[b]Lindow and Peterson (147) assert that on a dry basis, green leafy vegetables rank highest in Mn content.

B. Animals

Similarly to plants, the manganese content of animal tissues lies in the range of trace to minute. Manganese is distributed widely throughout body tissues and fluids with a tendency to be more prevalent in mitochondria-rich tissues. Here the concentration is greater in the mitochondria than in the cytoplasm or other organelles. There is also a high degree of association of manganese with melanins. Peterson and Skinner (201) present a general value for manganese concentration in

animal tissue of 1.0 ppm (range: 0.078-3.8 ppm, based on 13 samples, fresh basis). Because the manganese content is highly characteristic of certain organs (perhaps even more so than of certain animals), the authors feel no significance should be attached to this figure other than as an indicator of its presence in minute quantities. Fore and Morton (076) performed an extensive analysis of the tissues of the rabbit for manganese concentrations, results of which are shown in Table 2.

C. Synthetics

Manganese is used in steel alloys for improving rolling and forging qualities, as well as to impart strength, hardness and hardenability. Manganese is used to "decolorize" glass which is green due to iron impurities. Manganese may also be used to impart an amethyst color to glass.

No information was found in the literature which discusses the importance of containers or utensils containing manganese which come into contact with food.

D. Natural inorganic sources

Manganese occurs in water as a result of soluble minerals. Untreated groundwater may contain as much as 2 mg/liter, primarily as the dissolved carbonate. The amount of dissolved manganese decreases in water of higher alkalinity and of higher carbonate level (185).

Table 2

Manganese in Rabbit Tissues (076)

Tissue	Fresh tissue wt. (g.)	Dry matter (%)	Ash as % of fresh tissue	Ash as % of dry tissue	Manganese content in p.p.m. in Fresh tissue	Manganese content in p.p.m. in Dried tissue	Manganese content in p.p.m. in Ash
Pituitary	0·0078	12	—	—	3·9	31	—
Pituitary	0·0071	14	—	—	1·4 ; 2·4	9·7 ; 17	—
Pituitary	0·0063	24	—	—	1·8	7·6	—
Grey hair	0·713	90	1·5	1·6	0·99	1·1	67
White hair	0·816	87	0·56	0·65	0·98	1·1	170
Hide	1·32	46	0·72	1·6	0·38	0·82	53
Fascia	4·97	23	1·1	4·7	0·024	0·10	2·2
Spleen	1·51	22	1·0	4·5	0·22	0·97	21
Bone marrow	0·630	53	0·99	1·8	0·045	0·087	4·7
Ileum	0·723	18	1·1	6·0	1·7	9·6	160
Kidney (one)	7·11	26	1·3	4·9	1·2	4·7	97
Duodenum	1·41	20	1·2	6·1	1·1	5·6	92
Mucosa	1·03	18	1·2	6·8	1·6	8·6	130
Muscularis	2·22	17	0·91	5·5	0·50	3·1	55
Salivary gland	1·42	22	1·6	7·1	1·4	6·2	88
Stomach	1·75	23	1·1	4·7	1·0	4·4	95
Cæcum	1·08	17	0·79	4·7	0·82	4·9	100
Bile	1·61	20	1·7	8·5	0·48	2·4	28
Heart	1·06	22	1·0	4·5	0·28	1·3	28
Liver	3·84	30	1·6	5·3	2·0	6·7	130
Liver	3·03	29	1·5	5·3	2·2 ; 2·1	7·6 ; 7·9	140 ; 160
Liver	2·79	24	1·2	4·9	2·0	8·4	170
Liver	3·29	25	1·2	4·9	2·2	8·7	180
Pancreas	0·225	74	1·1	1·5	1·6	2·1	140
Pancreas	0·297	26	1·5	5·8	1·6 ; 1·6	6·2 ; 4·2	110 ; 130
Adrenals (two)	0·342	49	1·5	3·1	0·67	1·3	44
Cortex	0·290	42	1·1	2·7	0·56	1·3	50
Medulla	0·027	39	—	—	0·60	1·5	—
Ovaries (two)	0·681	23	1·5	6·3	0·60	2·6	41
Mammary gland	1·88	62	0·31	0·50	0·25	0·41	82
Mammary gland (lactating)	0·738	45	1·2	2·7	2·2	4·8	180
Brain	2·75	22	1·5	6·9	0·36	1·6	24
Muscle	2·07	23	1·2	5·0	0·13	0·56	11
Pineal	0·0061	15	—	—	3·8 ; 2·3	26 ; 15	—
Pineal	0·0018	—	—	—	0·83	3·3	—
Gall bladder	0·431	27	1·7	6·6	0·91	3·4	53
Nerve	0·178	41	1·4	3·5	0·086	0·21	6·1
Thyroid	0·0798	34	1·3	3·7	0·29	0·84	23
Thyroid	0·122	33	0·25	0·75	0·21 ; 0·24	0·64 ; 0·76	85 ; 42
Thyroid	0·112	26	1·3	4·7	0·21	0·79	17
Testes (two)	0·0421	32	—	—	0·36	1·2	—
Spinal cord	0·393	31	1·7	5·5	0·39	1·3	23
Lung	0·868	18	0·99	5·4	0·011	0·061	1·1
Thymus	0·018	67	—	—	0·45	0·67	—

[Analysis by diethyaniline method of Fore and Morton (077).]

BIOLOGICAL DATA

I. Acute Toxicity

Introduction

Schroeder et al. (222) pointed out in their manganese review that divalent manganese has a low order of toxicity to living organisms. As Table 3 shows, vertebrates can tolerate large concentrations of this manganese ion (Mn^{2+}). They further noted that of all the trace metals tested, manganese is the least toxic to fish with the exception of iron. Heptavalent manganese, which is not found in nature, is apparently extremely toxic. This observation is cited as illustrative of the generalization that the natural valences of an element on the surface of the earth are the least toxic forms.

Handovsky et al. (092) concluded from their experiments with mice, guinea pigs and rabbits (see this section A 1, C 1, and D 2) that a very high manganese concentration in the blood must be reached to cause acute poisoning. Lower concentrations caused liver damage.

A. Mice

1. Handovsky et al. (092) found that mice (strain, age, number and sex not given) died within 12 hours maximum following subcutaneous injection of 50 mg/kg BW of either manganese chloride ($MnCl_2$) or manganese citrate [$Mn_3(C_6H_5O_7)_2$]. The toxic symptoms started with paralysis in the hind legs which advanced to involve the exterior limbs. The animals died of asphyxial seizures. It was found that in chronic toxicity experiments the citrate was much more poisonous than the chloride.

Table 3. Acute Toxicity Data

Substance	Animal (Species)	Sex & No. (M or F)	Route (p.o.,l.v.,s.c., l.p.,l.m., other)	Dosage mg/kg body wt.	Measurement (LD50, ED50 or other)	Ref. Bibliogr. No.
Manganous ion (Mn^{2+})	Daphnia Magna, fresh water	not given	water	p.p.m 50	LD_{100}	Schroeder et al. (222)
	flatworms	not given	water	700	LD_{100}	Schroeder et al. (222)
	fish, fresh water	not given	water	2420-3450	LD_{100}	Schroeder et al. (222)
	birds	not given	water	4800	LD_{100}	Schroeder et al. (222)
	chicks	not given	water	4800	LD_{100}	Schroeder et al. (222)
	rats	not given	water	> 2000	LD_{100}	Schroeder et al. (222)
	rabbits	not given	water	1250-6000	LD_{100}	Schroeder et al. (222)
	pigs	not given	water	500-2000	LD_{100}	Schroeder et al. (222)
	lambs	not given	water	5000	LD_{100}	Schroeder et al. (222)

Table 3 (cont.)

Substance	Animal (Species)	Sex & No. (M or F)	Route (p.o., i.v., s.c., i.p., i.m., other)	Dosage mg/kg body wt.	Measurement (LD50, ED50 or other)	Ref. Bibliogr. No.
Manganese Carbonate ($MnCO_3$)	rabbits	27 (sex not given)	i.v.	21.3 mg/kg Mn	LD_{100}	Sabbatini (219)
Manganese Citrate [$Mn_3(C_6H_5O_7)_2$]	mice	not given	s.c.	50	LD_{100}	Handovsky et al. (092)
	guinea pigs	not given	s.c.	50	LD_{100}	Handovsky et al. (092)
	rabbits	not given	s.c.	50	LD_{100}	Handovsky et al. (092)

Table 3 (cont.)

Substance	Animal (Species)	Sex & No. (M or F)	Route (p.o.,i.v.,s.c., i.p.,i.m., other)	Dosage mg/kg body wt.	Measurement (LD50, ED50 or other)	Ref. Bibliogr. No.
Manganese Chloride ($MnCl_2$)	fish	not given	-	300mg Mn/liter	LD_{100}	Von Oettingen (269)
	mice	not given	s.c.	50	LD_{100}	Handovsky et al. (092)
	rats	6 (sex not given)	s.c.	50	LD_{100}	Mackiewicz (152)
	rats	6 (sex not given)	s.c.	60	LD_{100}	Mackiewicz (152)
	guinea pigs	not given	s.c.	50	LD_{100}	Handovsky et al. (092)
	rabbits	not given	s.c.	50	LD_{100}	Handovsky et al. (092)
	rabbits	not given	i.v.	18mg Mn/kg	LD_{100}	Cervinka (035)
	rabbits	25 (sex not given)	i.v.	21.4mg Mn/kg	LD_{100}	Sabbatini (219)
	dogs	not given	i.v.	56mg Mn/kg	LD_{100}	Cervinka (035)

Table 3 (cont.)

Substance	Animal (Species)	Sex & No. (M or F)	Route (p.o.,l.v.,s.c., l.p.,l.m., other)	Dosage mg/kg body wt.	Measurement (LD50, ED50 or other)	Ref. Bibliogr. No.
Manganese Oxide (MnO)	guinea pigs	not given	s.c.	28-30	LD_{100}	Von Oettingen (269)
	rabbits	not given	s.c.	12-13	LD_{100}	Von Oettingen (269)
	cats	not given	s.c.	6-8 13-14	LD_{100} (2 days) LD_{100} (24 hrs)	Von Oettingen (269)
	dogs	not given	s.c.	6-8 13-14	LD_{100} (2 days) LD_{100} (24 hrs)	Von Oettingen (269)
Manganese Phosphate, colloidal $[Mn_3(PO_4)_2]$	rabbits	16 (sex not given)	i.v.	23mg/kg Mn	LD_{100}	Sabbatini (219)
Manganese Sulfate ($MnSO_4$)	mice	54 M	i.p.	534 (479-595 at 95% confidence limit)	LD_{50} (1 hour)	Ceasar and Schnieden (034)

2. Ceasar and Schnieden (034) determined the one-hour LD_{50} for manganese sulfate ($MnSO_4$) in 54 male, albino mice (TT strain, 17-30g) by intraperitoneal injection. It was found to be 534 mg/kg BW (95% confidence limit, 479-595).

B. Rats

Mackiewicz (152) subcutaneously injected 36 two-month old rats which were divided into six groups of six animals, with the following doses of $MnCl_2$ for four weeks; 10, 20, 30, 40, 50 and 60 mg/kg BW. At the two lowest doses (10 and 20 mg/kg BW) no effect was observed. A reduction in weight gain was noted at 30 mg/kg BW and at 40 mg/kg BW; in addition to reduced weight gain one out of six animals died. The LD_{50} dose was found to be 50 mg/kg BW and the LD_{100} was 60 mg/kg BW.

C. Guinea Pigs

1. Handovsky et al. (092) also found that guinea pigs (age, number and sex not given) subcutaneously injected with 50 mg/kg BW manganese chloride or citrate died within 12 hours. The symptoms were similar to those seen with mice (see this section, A 1).

2. Von Oettingen (269) cited Kobert's study with manganese oxide (MnO) in 1883, in which it was found that a subcutaneous injection of 28-30 mg/kg BW was fatal to guinea pigs. During the first few hours following administration no symptoms were observed. Later the animals developed convulsions followed by paralysis. When smaller doses were given the animals died from "progressive depression".

D. Rabbits

1. Von Oettingen (269) also cited Kobert's finding that subcutaneous injection of 12-13 mg/kg MnO was fatal to rabbits. The same progression of symptoms was seen as with guinea pigs (see this section, C 2).

2. Handovsky et al. (092) found that rabbits (age, number and sex not given) subcutaneously injected with 50 mg/kg BW manganese chloride or citrate died within 12 hours. The symptoms of toxicity were similar to those seen with mice (see this section, A 1).

3. Cervinka (035) found that the average lethal dose when $MnCl_2$ mixed with sodium thiosulfate (amount not stated) was injected intravenously into rabbits was 18 mg Mn/kg BW. The author suggested that an explanation for the symptoms observed in both chronic and acute poisoning, an upset of the digestive and urinary functions, is that there is a paralysis of the sympathetic nervous system which brings about an upset of the digestive organs, an increase in urinary output, the lowering of the blood pressure and the slowing and deepening of the cardiac function. A fall in temperature as low as 2° was also observed in rabbits. At autopsy acute catarrh, inflammation of the intestine and occasionally nephritis were observed (for further information see this section, E 2).

4. Sabbatini (219) determined the lethal doses for $MnCl_2$, manganese carbonate ($MnCO_3$) and colloidal manganese phosphate [$Mn_3(PO_4)_2$] with rabbits. The $MnCl_2$ solution (about 1.37 g Mn/liter) was injected intravenously into 23 rabbits (900-1200 g, sex not given). The minimum lethal dose was found to be 21.4 mg/kg BW of Mn. The author attributed death to the effect of the $MnCl_2$ on the heart. Following the injection, the heart beat frequency slowed down, the arterial pressure was reduced and finally heart arrest took place. Immediate dissection showed the heart arrested in diastole with only slight fibrillating movements occurring. At death there were convulsions and general paralysis.

Because of difficulties encountered when making a $MnCO_3$ solution, the author injected mixtures made from equal volumes of $MnCl_2$ and CO_2 prepared a few minutes before administration. The solution (about 1.37 g Mn/liter) was injected intravenously into 27 rabbits (900-1800 g, sex not given). The minimum lethal dose was found to be 21.3 mg/kg BW of Mn. The symptoms seen either in immediate or delayed death (20-48 hours) were the same as those described above for $MnCl_2$.

A solution of colloidal $Mn_3(PO_4)_2$ (about 1.37 g Mn/liter) was intravenously injected into 16 rabbits (900-1300 g, sex not given). The minimum lethal dose, 23 mg/kg BW of Mn, caused death anywhere from a few minutes after injection up to 19 hours. The symptoms observed were the same as those described above for $MnCl_2$.

E. Dogs

1. Von Oettingen (269) cited Kobert's study with MnO in 1883, in which it was found that with subcutaneous injections, 13-14 mg/kg BW is lethal to dogs in 24 hours and 6-8 mg/kg BW is lethal in two days. No further details were given.

2. Cervinka (035) found that the average lethal dose of $MnCl_2$ mixed with sodium thiosulfate (amount not stated) was 56 mg Mn/kg BW. He found that the heart is the first organ affected. The fall in blood pressure appeared at almost the same time as cardiac paralysis. In the final phase of intoxication the cardiac contractions resembled those produced by fainting, the blood pressure falls and the breathing becomes increasingly shallow. Repeated injection of the toxic dose led to weakening, prostration, loss of weight and diarrhea (for further information see this section, D 3).

II. Short-Term Toxicity

Introduction

According to Underwood (262) of all the trace elements, manganese is the least toxic to birds and mammals. Excess manganese has apparently little or no effect on growth. The adverse effects of excess manganese on growth which have been reported appear to be caused by appetite depression. However, a relationship between manganese, iron metabolism and hemoglobin formation has been made evident (096). Changes in the thyroid and adrenal cortex have also been reported (145).

Mikhaylov (170) stated in his review on the pathogenesis of manganese poisoning that chemical and experimental observations indicate that following manganese toxicity the greatest changes take place in the basal ganglia where the degenerative and dystrophic alterations in the cells are more pronounced than elsewhere in the brain. Research has shown that exposure to manganese may give rise to the accumulation of acetylcholine or similar substances in the cortical synapses and peripheral myoneural synapses thus impairing synaptic conduction. The increased acetylcholine concentration, as pointed out by the author, is responsible for the subsequent blocking of the transmission of excitation, asynapsia, a process most pronounced in the basal ganglia but also found in the cerebral cortex.

A. Mice

1. Tal and Guggenheim (253) studied the effects of ingesting a magnesium salt on the calcification of mouse bone. Sixteen groups of 24, three-week old, male, Swiss mice (10-13 g) were maintained on diets of raw meat supplemented with manganese sulfate ($MnSO_4$) in various amounts

(2.5, 5.0, 10.0, or 25.0 mg/kg of meat) for two, four or six weeks. One group of controls received unsupplemented meat and one group of positive controls were given the meat supplemented with 3.6 g/kg calcium carbonate ($CaCO_3$). The results (given as means ± S.E.M.) at two, four and six weeks are summarized on Tables 4, 5, 6 respectively.

The significant observations were:

a. Diet maintained for two weeks (Table 4): (1) Supplemental manganese did not increase weight as much as supplemental calcium. (2) The manganese content of ash and bone was increased by both 10 and 25 mg/kg supplements. (3) Bone calcification was considerably improved with the 25 mg/kg manganese supplement.

b. Diet maintained for four weeks (Table 5): (1) The two smallest manganese supplements (2.5 and 5.0 mg/kg of meat produced a small weight increase, but there was no effect on weight with the larger amounts (10.0 and 25.0 mg/kg of meat). (2) All four of the different manganese supplements resulted in the same bone manganese concentration. (3) Calcification improved to a small degree with smaller manganese supplements but not with larger. (4) The calcium and phosphorus content of the bones when the largest supplement (25 mg/kg manganese) was given was significantly lower than with the two lower supplements (2.5 or 5.0 mg/kg).

c. Diet maintained for six weeks (Table 6): (1) The bone manganese content was similar to that at four weeks. (2) At the higher manganese supplementation (10.0 and 25.0 mg/kg of meat), growth was depressed. Bone ash and calcium and phosphorus contents were decreased. (3) The incorporation of radioactive calcium in

Table 4. Composition of Bones and Uptake of Radioactive Calcium by Femurs of Mice Maintained for 2 Weeks on A Meat Diet (253)

Supplement ...	None	10·0 mg. of Mn/kg. of meat	25·0 mg. of Mn/kg. of meat	3600 mg. of Ca/kg. of meat	No. of pairs of femurs in each sample	No. of samples in each mean
Weight increase (g.)	4·6 ± 1·7	6·5 ± 0·8	6·5 ± 1·0	7·8 ± 0·9	—	24
Weight of femurs (mg.)	98 ± 5·5	77 ± 5·3	85 ± 3·5	98 ± 5·6	1	24
Ash:						
(mg.)	13·7 ± 1·3	16·5 ± 1·1	20·2 ± 1·2	28·4 ± 2·2	1	24
(% of dry fat-free bone)	42·1 ± 0·63	42·2 ± 1·55	48·8 ± 0·49	53·6 ± 0·71	1	24
Ca:						
(% of ash)	30·4 ± 0·21	29·5 ± 0·15	32·1 ± 0·14	35·1 ± 0·10	1	24
(% of dry fat-free bone)	12·7 ± 0·18	12·4 ± 0·20	15·6 ± 0·22	18·0 ± 0·23	1	25
Ca incorporated:						
(μg./g. of dry fat-free bone)	78 ± 0·55	80 ± 0·53	77 ± 0·66	56 ± 0·21	1	6
(μg./100 mg. of bone Ca)	62 ± 0·22	64 ± 0·32	53 ± 0·48	30 ± 0·53	1	6
P:						
(% of ash)	15·7 ± 0·17	14·8 ± 0·10	16·3 ± 0·12	17·7 ± 0·15	3	6
(% of dry fat-free bone)	6·6 ± 0·12	6·3 ± 0·15	8·0 ± 0·13	9·6 ± 0·17	3	6
Mn:						
(mg./100 g. of ash)	490 ± 14	788 ± 12	811 ± 13	411 ± 12	3	6
(mg./100 g. of dry fat-free bone)	203 ± 27	332 ± 22	389 ± 24	219 ± 22	3	6

Table 5. Composition of Bones and Uptake of Radioactive Calcium by Femurs of Mice Maintained for 4 Weeks on A Meat Diet (253)

Supplement ...	None	2·5 mg. of Mn/kg. of meat	5·0 mg. of Mn/kg. of meat	10·0 mg. of Mn/kg. of meat	25·0 mg. of Mn/kg. of meat	3600 mg. of Ca/kg. of meat	No. of pairs of femurs in each sample	No. of samples in each mean
Weight increase (g.)	4·4 ± 1·3	7·0 ± 1·2	6·0 ± 0·9	3·9 ± 0·8	4·2 ± 0·8	9·3 ± 1·5	—	24
Weight of femurs (mg.)	97 ± 4·9	107 ± 9·0	97 ± 9·7	95 ± 7·3	111 ± 8·2	129 ± 9·5	1	24
Ash:								
(mg.)	19·0 ± 3·6	23·9 ± 1·8	21·1 ± 1·7	20·9 ± 3·2	23·0 ± 5·0	43·5 ± 3·6	1	24
(% of dry fat-free bone)	40·8 ± 0·48	48·1 ± 0·45	47·0 ± 0·57	45·4 ± 0·86	43·1 ± 0·93	59·0 ± 1·10	1	24
Ca:								
(% of ash)	28·9 ± 0·15	32·3 ± 0·22	32·3 ± 0·17	32·5 ± 0·16	31·3 ± 0·16	37·4 ± 0·18	1	24
(% of dry fat-free bone)	11·6 ± 0·18	15·4 ± 0·20	15·3 ± 0·10	14·7 ± 0·12	13·6 ± 0·15	22·6 ± 0·19	1	24
Ca incorporated:								
(μg./g. of dry fat-free bone)	73 ± 0·43	67 ± 0·41	70 ± 0·53	61 ± 0·38	67 ± 0·44	44 ± 0·59	1	6
(μg./100 mg. of bone Ca)	62 ± 0·53	42 ± 0·39	46 ± 0·40	40 ± 0·52	47 ± 0·35	20 ± 0·38	1	6
P:								
(% of ash)	15·0 ± 0·18	16·7 ± 0·12	16·6 ± 0·15	15·9 ± 0·13	15·7 ± 0·14	18·8 ± 0·19	3	6
(% of dry fat-free bone)	6·0 ± 0·13	8·5 ± 0·15	7·8 ± 0·17	7·1 ± 0·18	7·0 ± 0·11	11·5 ± 0·16	3	6
Mn:								
(mg./100 g. of ash)	474 ± 13	861 ± 19	904 ± 18	900 ± 24	956 ± 18	404 ± 20	3	6
(mg./100 g. of dry fat-free bone)	192 ± 15	450 ± 12	440 ± 20	410 ± 14	398 ± 12	243 ± 11	3	6

Table 6. Composition of Bones and Uptake of Radioactive Calcium by Femurs of Mice Maintained for 6 Weeks on A Meat Diet (253)

Supplement ...	None	2·5 mg. of Mn/kg. of meat	5·0 mg. of Mn/kg. of meat	10·0 mg. of Mn/kg. of meat	25·0 mg. of Mn/kg. of meat	3000 mg. of Ca/kg. of meat	No. of pairs of femurs in each sample	No. of samples in each mean
Weight increase (g.)	5·4 ± 1·4	6·9 ± 1·1	7·1 ± 1·0	6·9 ± 0·6	4·2 ± 0·8	13·5 ± 1·0	—	24
Weight of femurs (mg.)	98 ± 9·2	113 ± 5·5	109 ± 5·2	104 ± 6·8	111 ± 5·7	141 ± 4·4	1	24
Ash:								
(mg.)	19·8 ± 1·7	24·2 ± 1·4	22·7 ± 1·8	19·6 ± 1·3	20·0 ± 0·7	44·0 ± 1·6	1	24
(% of dry fat-free bone)	44·3 ± 0·41	46·1 ± 0·63	45·6 ± 0·58	39·9 ± 0·50	37·1 ± 0·65	59·3 ± 0·73	1	24
Ca:								
(% of ash)	32·9 ± 0·29	32·3 ± 0·37	31·4 ± 0·20	30·7 ± 0·21	28·8 ± 0·28	41·2 ± 0·20	1	24
(% of dry fat-free bone)	14·5 ± 0·19	15·0 ± 0·36	14·0 ± 0·24	12·1 ± 0·20	10·7 ± 0·13	24·5 ± 0·20	1	24
Ca incorporated:								
(μg./g. of dry fat-free bone)	72 ± 0·20	67 ± 0·21	67 ± 0·33	64 ± 0·29	70 ± 0·20	39 ± 0·72	1	6
(μg./100 mg. of bone Ca)	50 ± 0·29	45 ± 0·11	47 ± 0·28	52 ± 0·38	63 ± 0·13	17 ± 0·55	1	6
P:								
(% of ash)	17·6 ± 0·42	17·5 ± 0·28	16·8 ± 0·12	16·2 ± 0·12	15·0 ± 0·09	20·9 ± 0·21	3	6
(% of dry fat-free bone)	7·6 ± 0·30	8·0 ± 0·25	7·8 ± 0·13	6·2 ± 0·11	5·8 ± 0·16	12·2 ± 0·43	3	6
Mn:								
(mg./100 g. of ash)	530 ± 10	990 ± 12	915 ± 13	980 ± 14	1087 ± 24	475 ± 4	3	6
(mg./100 g. of dry fat-free bone)	236 ± 19	456 ± 14	432 ± 22	381 ± 33	398 ± 28	275 ± 12	3	6

bone calcium was increased at the highest supplementation.

The authors concluded that relatively large doses of manganese (as $MnSO_4$) administered over a prolonged period were toxic and induced defective bone calcification.

B. Rats

1. Findlay (071) investigated the histological changes produced by manganese chloride ($MnCl_2$) in animals. Two experiments were carried out with rats. (Strain, age and sex not given.) In the first experiment, ten rats were injected subcutaneously with 3 mg $MnCl_2$ on alternate days. The experiment was carried out for a maximum of 86 days when the tenth rat died. The observations were:

a. Four rats died in the first four weeks from septic infections.

b. The remaining six rats showed varying degrees of fibrosis of the liver.

c. All the cirrhotic rats lost weight (25-37 g).

d. The six rats died from the 38th to the 84th day of the experiment.

A second experiment was carried out to determine whether a similar cirrhotic condition could be produced by feeding the manganese salt. The regular diet of 12 rats (strain, age, and sex not given) was supplemented with 0.3-0.4 g manganous chloride per day. The animals died within six to ten weeks. Up until death they appeared in good condition and some gained weight.

It was observed that:

a. All the animals showed some degree of liver cirrhosis.

b. Histologically they were similar to the injected rats of the first experiment.

c. Jaundice was noted in all post-mortem examinations.

d. Three of the animals had rigid limbs for several days preceding death.

The livers of the control rats (number not stated) were completely free of fibrosis. Similar experiments by the same researcher with guinea pigs and rabbits are described later in this section.

2. Becker and McCollum (012) studied the effects on growth and reproduction of feeding a manganese salt ($MnCl_2 \cdot 4H_2O$) to rats. Young rats (40-50 g, number and strain not stated) were fed a basal ration supplemented with $MnCl_2 \cdot 4H_2O$ added at the following levels: 0.18%, 0.36%, 0.9%, 1.8%, 3.6%, representing a dietary manganese

addition of: 0.0499, 0.0998, 0.2495, 0.4990 and 0.9980 g respectively. The animals were maintained on these diets for 240 days. It was observed that they grew well and reproduced healthy young on all but the highest manganese supplement (3.6%).

The authors pointed out that the basal ration they used contained high levels of calcium (0.63 g/100 g) and phosphorus (0.72 g/100 g). They attributed the prevention of symptoms of manganese toxicity to the high phosphorus level which reduced the amount of absorbable magnesium. They concluded that the controlling factor in determining the level at which manganese is toxic is the condition of the intestines which influences the absorbability of manganese.

3. Moinuddin and Lee (175) compared the effects of feeding three different sulfates, manganese, magnesium, and sodium, to rats. (Only the experiment using manganese sulfate ($MnSO_4$) is discussed here.) Four different diets were fed to groups of six weanling male Sprague-Dawley rats ad libitum along with water for four weeks. The four diets were: (1) The basal diet designated as the cornstarch diet to the controls. (2) Experiment a, in which $MnSO_4 \cdot H_2O$ was added at a level of 0.88 mmole/kg of feed (part of the dietary cornstarch was replaced by the added salt). (3) Experiment b, in which the same salt replaced dietary cornstarch as follows: added initially at 8.64 mmole/kg of feed; doubled on the ninth day to 17.28 mmole/kg; doubled again on the 17th day to 34.56 mmole/kg of feed; and again on the 25th day to 69.12 mmole/kg of feed where it was maintained until the end of the experiment. The final percentage by weight in the diet was 1.2%. (4) Experiment c, in which the $MnSO_4 \cdot H_2O$ was added, replacing

some of the cornstarch, at a level of 138 mmole/kg of feed for the entire experiment.

Since this was a comparative study, the results were stated relative to the other two salts tested. At the low levels of salt administration in experiments a and b, there were no significant differences noted. The following observations therefore, refer to experiment c. Compared to rats of the other feeding groups, the rats fed manganese sulfate: (a) ate less feed, (b) gained less body weight, (c) required more feed per gram of gain, (d) drank more water and voided more urine, (e) showed depigmentation of the labial surfaces of the incisor teeth, (f) had less hemoglobin and a higher erythrocyte count, and (g) had less serum inorganic phosphorus.

The authors attributed the incisor depigmentation to lowered plasma and body stores of iron owing to an excess of dietary manganese. They also noted that manganese excess had been reported to cause a decrease in hemoglobin and an increase in red blood cell count in other species.

4. Levina and Minkina (145) investigated the state of the adrenal cortex in animals poisoned with two manganese oxides (MnO and MnO_2). The authors noted in their introduction that in rabbits, manganous chloride is known to cause hyperemia, hemorrhages, degenerative and necrotic changes in the adrenals and an increased quantity of lipids in the cortical layer, when administered either with food or by subcutaneous injection (the source of these observations is not given). The authors also mentioned a previous finding of their own that in rats poisoned with manganese oxides (route of administration not stated),

the thyroid shrank and its functioning was depressed.

Three groups of 15 white rats each (sex, age and weight not given) were subcutaneously injected respectively with either 100 mg/kg BW MnO_2 or MnO suspended in physiological saline or physiological saline alone (controls), every other day for about one month (a total of 18 injections). During the course of the experiment the animals given the manganese oxides showed the toxic symptoms of weight loss, adynamia and in some, central paresis of the hind legs.

After sacrifice the adrenals of the experimental animals were found to have an increased size and weight compared with the controls. Histochemical studies showed the quantity of cortical lipids to be substantially decreased. In general the adrenal cortex in the experimental animals showed a marked depletion of ketosteroids, the amount of ascorbic acid decreased substantially, and the RNA content decreased slightly. These changes were more pronounced with MnO_2 than with MnO.

The authors pointed out that: (a) The above changes presented a picture of increased adrenocortical function. (b) Manganese is known to produce lesions in the subcortical region of the brain. (c) When thyroid changes resulting from manganese oxide toxicity are also taken into consideration, the changes in the adrenal cortex could be considered as manifestations of impairment of neurohumoral regulation. (d) After prolonged exposure to these toxic manganese oxides, the above picture of adrenocortical stimulation could change to one of inhibition of adrenocortical function.

5. Baxter et al. (010) studied some effects of an acute manganese load on rats. Manganous chloride diluted in normal saline to give dose

levels of from 5 to 150 mg manganese was administered subcutaneously to albino Holtzman rats (100 to 500 g, number not stated). Controls received normal saline only.

At various times between 1 and 72 hours following the $MnCl_2$ administration blood samples were taken. It was found that in animals receiving 15 mg/100g BW, blood hemoglobin, hematocrit and erythrocyte mean corpuscular volume increased within 10 hours following the injection, peaking between 12 to 18 hours. Serum chloride, phosphorus and magnesium increased while serum calcium and iron were markedly lowered (see Table 7).

At 18 hours following administration of 17 mg/100g BW or more of manganese, necrotic changes were seen in hepatic tissue which were most pronounced in the periphery of the hepatic lobule.

The following were observed after administration of 30 mg/100g BW manganese: (a) Peripheral blood smears showed anisocytosis, basophilic stippling, and hypochromia of red blood cells. (b) Microscropic examination disclosed apparent increase in liver and spleen iron content in several rats 48 hours after the acute dose was given.

The authors postulated that this increase in hepatic and splenic iron content, which is concurrent with the fall in serum values, suggested the possibility of an intracellular shift of the metal.

6. Chandra and Srivastava (036) undertook the study of the production of early brain lesions in rats by manganese. Male albino rats (I.T.R.C. colony, 150 g average weight) were divided into two groups: Group I, 30 control animals, were injected intraperitoneally with 0.5 ml distilled water; Group II, 60 experimental animals were injected

Table 7

Alterations in Blood Hemogram and Chemistry Values 20 Hours Following Acute Manganese Administration (15 mg/100 g Body Weight). (010)

	Controls		Mn injected	
Hgb	13.7 ± .9	g%	16.3 ± 1.1	p<.001
Het	47.7 ± 2.8	%	53.9 ± 2.9	p<.001
RBC	6.97 ± 1.90	($X10^6$) cells/mm^3	6.66 ± 1.99	($X10^6$)
M.C.V.	65.0 ± 2.5	μ^3	72.8 ± 3.2	p<.001
M.C.H.	20.5	$\mu\mu g$	22.6	
M.C.H.C.	32.0	g %	31.0	
Serum protein	5.65	g %	5.77	
Blood volume	22.0	ml	23.0	
Plasma volume	11.8	ml	11.5	
Serum Mg	2.36 ± .39	mg %	3.29 ± .60	p<.01
Serum Cl	104 ± 4	mEq/l	123 ± 6	p<.001
Serum P	2.64 ± .16	mg %	3.26 ± .24	p<.01
Serum Ca	4.53 ± .60	mEq/l	3.14 ± .38	p<.001
Serum Fe	3.62 ± 1.02	µg/ml	.81 ± .20	p<.001

intraperitoneally daily for 180 days with an aqueous solution of $MnCl_2$ (8 mg/kg).

At 30 day intervals up to 180 days, four animals from Group I and six from Group II were sacrificed. No unusual neurological symptoms were developed by the animals during this period. Up to 120 days no gross pathological changes were noted in the brain and spinal cord. At 150 and 180 days, the brain appeared paler than normal.

Microscopic examination showed the following: (a) No pathological changes in Group I animals (controls). (b) Up to 90 days, no pathological changes in the experimental animals. (c) At 120 days, scattered neuronal degeneration in the cerebellar and cerebral cortex was seen. (d) At 150 days, vacuolated nerve cells in the cerebral and cerebellar cortex and other degenerative changes appeared. (e) At 180 days, there was a fairly large number of degenerated neurons scattered in the cortex. (f) The nerve cell count at various intervals (see Table 8) showed an increase after 90 days of treatment. This indicated that manganese-induced degenerative brain lesions were present at 120 days and increased in severity with time, reaching a maximum at 180 days.

Thus the maximum number of degenerated neurons were present when the amount of manganese in the brain was at a maximum (see Table 9).

The authors concluded that: (a) the extent of damage to the brain cells is directly related to the amount of manganese present in the brain; (b) the period up to 120 days is apparently the threshold for the appearance of microscopic lesions; and (c) the experiment supports the hypothesis that damage to the brain cells of experimental animals can be caused by soluble manganese.

Table 8

Average Number of Degenerated Neurons in The Cortex of Brain in Control and Manganese Injected Rats (036)

Neurons	Control	Manganese injected rats					
		Days					
	0	30	60	90	120	150	180
Degenerated	54	54	53	50	173	319	433
Normal	946 (10)	946 (4)	947 (4)	950 (4)	827 (4)	681 (4)	567 (4)
Percent	5.4	5.4	5.3	5.0	17.3	31.9	43.3

Figures in brackets indicate number of animals.

Table 9

Manganese Content in Brain of Control and Manganese Injected Rats (036)

Manganese mg/g dry weight of brain tissue	Control	Manganese injected rats		
	0	60 days	120 days	180 days
Mean value ± S. E. M.	2.253 ± 0.2190 (7)	3.784 ± 0.2445 (6)	4.863 ± 0.1378 (6)	6.849 ± 0.1502 (6)
Statistical significance of the difference –				
1. from control ...	–	Significant $P < 0.05$	Highly significant $P < 0.01$	Highly significant $P < 0.01$
2. from 60 days .	–	–	Not significant	Highly significant $P < 0.01$
3. from 120 days .	–	–	–	Significant $P < 0.05$

Figures in brackets indicate number of animals.

C. Hamsters

Voigt and Saldeen (266) produced severe liver degeneration in hamsters with manganese sulfate ($MnSO_4$). A group of 60 Syrian golden hamsters (60-130 g, 3-9 mos.) were subcutaneously injected with an aqueous solution of 4.5 mg $MnSO_4$/100 g BW daily for 10 days. Two animals were sacrificed daily starting with one day after the beginning of the experiment. The liver degeneration was followed in detail starting with the appearance of large droplet fatty deposition in the parenchyma cells and continuing to icteric necroses emanating from the acinus periphery on the sixth or seventh day. The kidneys also showed necroses of the epithelial cells in the tubules and small necroses with agglomerations of polymorphonuclear leukocytes were seen in the myocardium.

D. Guinea Pigs

Findlay (071) investigated the histological changes produced by manganous chloride in guinea pigs because they are considered to be relatively less susceptible than rabbits to its toxic effects. Subcutaneous injections of an aqueous solution (3 mg $MnCl_2$ dissolved in water) were administered on alternate days (see Table 10 for further details). It was found that five of the experimental animals (one died prematurely of pneumonia) showed some degree of liver fibrosis. The fibrosis could be easily seen in the portal tract region and was associated with the proliferation of the smaller bile ducts. There was fatty degeneration in the liver parenchyma. The changes seen were similar to those found in rats and rabbits by the same investigator. Controls showed no fibrosis of the liver.

Table 10

Experiments on $MnCl_2$ Injected into Guinea Pigs (071)

	Weight of animal in gm. at beginning of experiment.	Weight of animal in gm. at end of experiment.	Period of survival in days.
1	285	220	12
2	380	350	29
3	300	310	32
4	290	225	34
5	270	270	39
6	260	270	44

E. Rabbits

1. Findlay (071) investigated the histological changes produced by $MnCl_2$ in animals. Three experiments were carried out with rabbits: one in which single subcutaneous injections of an aqueous solution of $MnCl_2$ in dosages from 10 to 60 mg were given to ten rabbits (see Table 11); and two experiments in which repeated subcutaneous injections of either 3 mg or 5 mg of aqueous $MnCl_2$ were administered every second day to young rabbits (see Table 12 for data concerning 5 mg dose). Control rabbits showed none of the pathological changes found in the internal organs of the experimental animals.

The main change produced by the injected $MnCl_2$ was a form of liver cirrhosis of the biliary type. In its earliest stages the fibrous overgrowth in the liver is confined to the periphery of the lobules in relation to the portal spaces, a monolobular cirrhosis. In its later stages, the lobules are entirely invaded by the freshly formed fibrous tissue. Jaundice may frequently be present as well.

The author postulated that this toxic effect of manganous chloride may be related to the fact that manganese salts are very largely

Table 11

Experiment on $MnCl_2$ Injected into Rabbits (Single Large Dose) (071)

	Weight of rabbit in gm.	Doses in mg.	Period of survival in hours.
1	1800	10	48
2	1450	15	40
3	1400	20	48
4	1550	25	36
5	1500	35	40
6	1250	40	42
7	1750	45	28
8	1450	50	22
9	1400	55	24
10	1850	60	26

Table 12

Experiment on $MnCl_2$ Injected into Rabbits Every Second Day (071)

	Weight in gm. at beginning of experiment.	Weight in gm. at end of experiment.	Date of death in days.
31	1520	1380	17
32	1440	1400	17
33	1100	1020	21
34	1220	1080	22
35	1450	1300	23
36	1540	1200	25
37	1400	1350	Suffering from coccidiosis.
38	1420	1400	31
39	1420	1280	35
40	1500	1200	35
41	1630	1640	21
42	1700	1480	26
43	1750	1730	38
44	1560	1430	58
45	1420	1400	2
46	1950	1980	45
47	1870	1920	Still alive.
48	1230	1200	42
49	1380	1420	35
50	1180	970	50
51	1310	1260	50
52	1150	1050	39

excreted by the bile.

2. Butt (032) investigated the effect of manganese chloride on the livers and kidneys of rabbits. Three different experiments were performed with young rabbits (6 mos; no other information given): (1) Ten rabbits were injected subcutaneously two or three times a week (see Table 13 for details) with manganese chloride. (2) Three rabbits were intravenously injected with manganese chloride (see Table 14 for details). (3) Five rabbits were fed manganese chloride daily with their food for 11 to 41 weeks (see Table 15 for details).

The results of the first experiment showed that the kidneys sustained the most constant and severe lesions of all the organs examined. Microscopically, the changes observed were: (a) marked fatty degeneration in the tubules; and (b) necrosis of the epithelial cells accompanied by indications of regeneration. Only one of the three rabbits in the second experiment showed a kidney lesion. None of the animals in the third experiment had damaged livers or kidneys. Twelve control rabbits showed no gross or microscopic lesions.

The author concluded that amyloid nephrosis was produced in rabbits by chronic poisoning from subcutaneous injections of manganese chloride. The amyloid degeneration was also occasionally found in the spleen.

Table 13

Rabbits Receiving Subcutaneous Injections of Manganese Chloride (032)

Rabbit	Duration of Experiment, Wk.	Number of Injections	Total $MnCl_2$ Received, Gm.	Albumin in Urine, Mg. per 24 Hr. Specimen at Termination of Experiment	Lesions of Kidney	Amyloid in Kidney	Lesions of Other Organs	Amyloid in Other Organs
1	12	27	0.062	.....	Cloudy swelling, tubular necrosis	None	Liver: areas of necrosis, small amount of pigment	None
2	12	27	0.062	.....	Cloudy swelling, tubular necrosis	None	Liver: areas of necrosis, small amount of pigment	None
3	12	27	0.062	.....	Cloudy swelling, tubular necrosis	None	Liver: areas of necrosis, small amount of pigment	None
4	34	89	0.482	1010.0	Marked tubular and glomerular involvement, moderate interstitial scarring	+++	Liver: areas of necrosis, slight increase of periportal fibrous tissue, terminal pericarditis	None
5	29	75	0.500	1094.0	Marked tubular and glomerular involvement, moderate interstitial scarring	+++	Spleen: pigment Liver: small amount of pigment	None
6	48	107	0.876	86.0	Moderate tubular and glomerular involvement	++	Lung: bronchopneumonia Liver: moderate increase in periportal fibrous tissue Spleen: pigment	None
7	48	103	0.929	110.0	Moderate tubular and glomerular involvement	++	Lung: bronchopneumonia Spleen: pigment	None
8	17(F) 8(S)	(17)F 13 S	1.050 0.154	1090.0	Marked tubular and glomerular involvement, moderate interstitial scarring	+++	Lung: hemorrhage Heart: interstitial scarring	Spleen
9	46	78	0.9768	120.9	Moderate tubular and glomerular involvement	++	Lung: hemorrhage	None
10	43	54	0.9054	1242.0	Marked tubular and glomerular involvement, moderate interstitial scarring	+++	Lung: hemorrhage	None

Table 14

Rabbits Receiving Intravenous Injections of Manganese Chloride (032)

Rabbit	Duration of Experiment, Wk.	Number of Injections	Total $MnCl_2$ Received, Mg.	Albumin in Urine, Mg. per 24 Hr at Termination of Experiment	Lesions of Kidney	Amyloid in Kidney	Lesions of Other Organs
11	10	18	0.242	1101.6	Marked tubular and glomerular necrosis	+	Spleen: pigment Liver: necrosis, small amount of pigment
12	42	79	0.5104	150.4	Marked cloudy swelling; otherwise normal	None	Spleen: pigment Liver: moderate amount of pigment
13	34	58	0.2620	156.6	Moderate cloudy swelling; otherwise normal	None	Spleen: pigment Liver: small amount of brown granular pigment

Table 15

Rabbits Fed Manganese Chloride (032)

Rabbit	Duration of Experiment, Wk.	Number of Feedings	Total $MnCl_2$ Received, Gm.	Albumin in Urine, Mg. per 24 Hr. at Termination of Experiment	Lesions of Kidney	Amyloid in Kidneys	Lesions of Other Organs
14	12	62	1.05	Test not done	None	None	Liver: necrosis
15	16	62	1.05	Test not done	None	None	Spleen: pigment
16	11	66	3.12	26.1	None	None	Liver: fatty infiltration
17	21	126	9.72	59.4	None	None	Liver: fatty infiltration Lung: bronchopneumonia
18	41	312	46.92	64.8	None	None	Liver: small amount pigment, fatty infiltration

3. Matrone et al. (158) determined the effect of excessive manganese (as $MnSO_4$) on hemoglobin regeneration in anemic rabbits. Anemia was produced in six 6-month old Dutch rabbits. Half were then fed a manganese supplemented diet and the other half, the controls, were fed only the diet. It was found that the hemoglobin regeneration, observed over a 6-week period was significantly greater in the control rabbits than in those fed 2000 ppm manganese (as $MnSO_4 \cdot H_2O$) with their diet. (See Table 16 for details of diets and Fig. 1 for hemoglobin change during the experiment).

Table 16

Diets Fed to Rabbits (158)

INGREDIENTS	CONTROL	MANGANESE [1]
	gm	*gm*
Chopped soybean hay	1600	1600
Glucose [2]	208	203
Casein (crude)	80	80
Wesson oil	112	112
Manganese mixture [3]	—	5

[1] Supplemented with 2000 p.p.m. of manganese.
[2] "Cerelose," Corn Products Refining Co., New York, N. Y.
[3] Contained 12.3 gm of $MnSO_4 \cdot H_2O$, mixed into 87.7 gm of glucose.

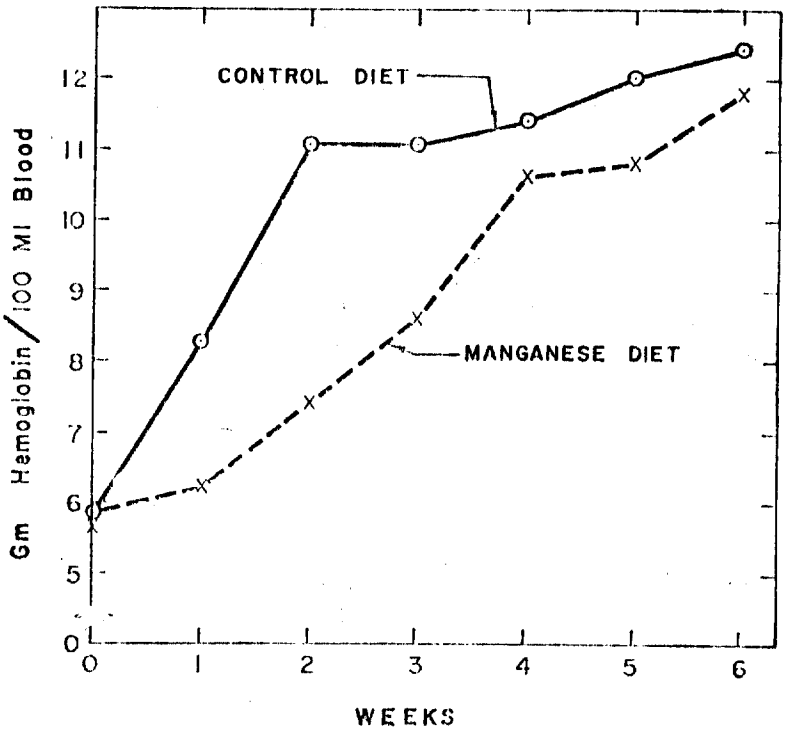


Fig. 1. Experiment 1, change in mean values of hemoglobin of rabbits fed the control and the manganese-supplemented diets during repletion.(158)

4. Umarji et al. (261) studied the effects of oral administration of manganese sulfate on the growth and manganese content of the fur of rabbits. Three groups of white rabbits (2000-2300 g), two per group were used in this 300-day experiment. Group I received 75 mg $MnSO_4$ daily in drinking water. Group II received 7 mg daily and Group III were the controls.

It was observed that the manganese content of the fur increased according to the dose. In Group I (75 mg/day), the manganese content of the fur was increased ten-fold. At this dose the animals developed anorexia, weight loss, anesthesia and paralysis of the hind paws. A decrease in the Mn content of the fur took place with the onset of

toxic symptoms. The average weight gains for the 300 days of the experiment were:

Experimental Group	dosage $MnSO_4$	Average weight gain
Controls	-	700 mg
I	75 mg/day	175 mg
II	7 mg/day	450 mg

The authors noted that their observation of a drop in the Mn content of the fur paralleled a similar observation in humans with manganese poisoning.

F. Dogs

Oettel (189) produced liver sclerosis in dogs with manganese chloride injections. Large dogs (age and sex not given) were injected intramuscularly with 20 mg/10 kg BW manganese chloride every two days. Two of the animals survived four weeks, two survived 8 weeks, and two for 16 weeks. A type of cholangitis-like cirrhosis with congestion of the portal vein developed by ten weeks.

G. Lambs

Hartman et al. (096) investigated the effects of manganese (as $MnSO_4 \cdot H_2O$) on hemoglobin formation in young, growing lambs. Two experiments were carried out in which young lambs were iron depleted either by an iron deficient diet or a combination of diet and phlebotomy. In one experiment the experimental animals, eight ewe and eight wether lambs (7 to 19 days old) were fed experimental diets supplemented with

various levels of manganese as the sulfate salt, $MnSO_4 \cdot H_2O$, (see Table 17 for details), after a preliminary period in which they were fed only fresh cow's milk.

Table 17

Levels of Manganese Added to The Basal Diet at Different Periods in The Experiment (096)

PERIODS		MANGANESE SUPPLEMENTATION [1]			
No.	Weeks	Mn_0	Mn_1	Mn_2	Mn_3
		ppm	*ppm*	*ppm*	*ppm*
1	0 – 12	0	5	15	45
2	12 – 20	0	5	30	90
3	20 – 24	0	–	450	900
4	24 – 35	0	–	2500	5000

[1] Supplements of manganese ($MnSO_4 \cdot H_2O$) were added to the whole milk diets on the basis of the total solids content.

The results shown on Figs. 2 and 3 were:

a. The mean biweekly hemoglobin concentrations were lowest for those experimental animals receiving the highest level of Mn supplementation (Mn_3) but a level as low as 45 ppm also caused a decrease in hemoglobin concentration.

b. The serum iron concentration also decreased the most for the animals on the highest Mn supplementation, but there a decrease even at the lowest level (45 ppm).

c. Decreased concentrations of iron in the liver, spleen and kidney were found at the highest supplementary level of manganese (5000 ppm).

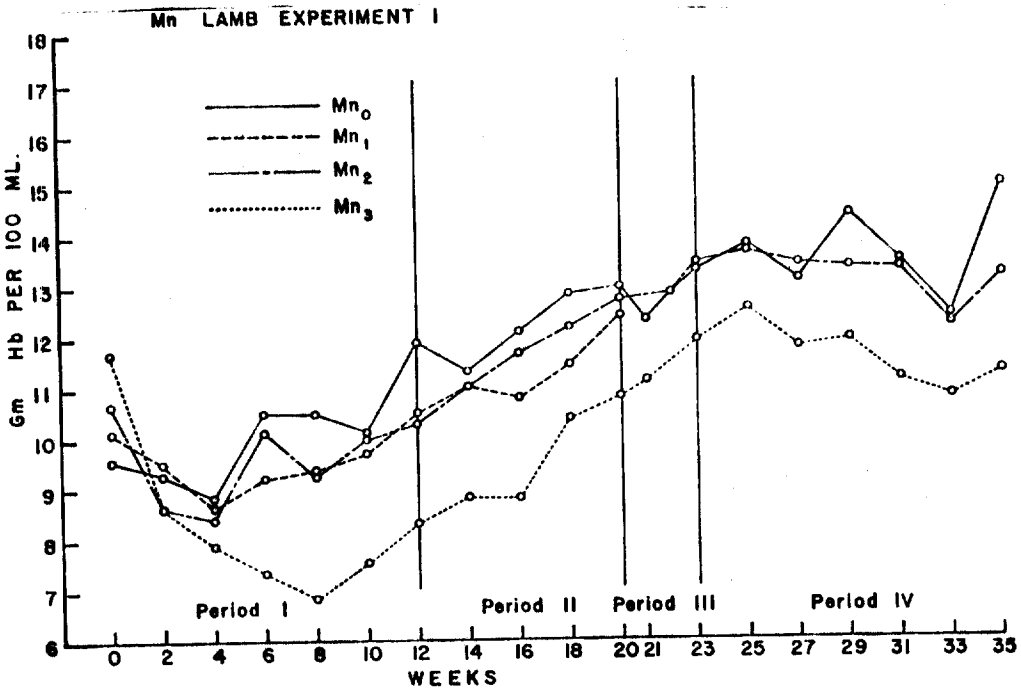


Fig. 2. Mean values of hemoglobin of the lambs fed various levels of manganese during the 4 periods. (096)

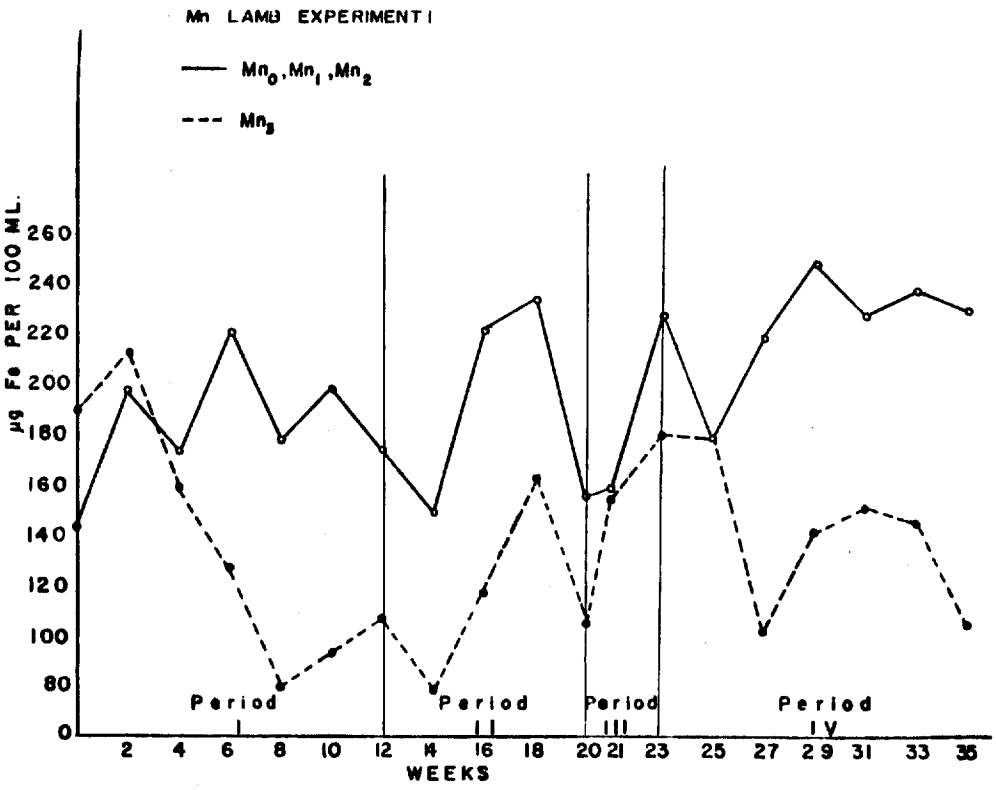


Fig. 3. Mean values of serum iron of the lambs fed various levels of manganese during the 4 periods. Since the responses to the Mn_0, Mn_1 and Mn_2 were not significantly different, the serum iron values were combined into one curve. (096)

In a second experiment, 8 lambs (6-9 days old) were fed cow's milk (2 months) and bled periodically (during the second month) prior to being put on a roughage basal diet supplemented with either 1000 ppm or 2000 ppm of Mn (as $MnSO_4 \cdot H_2O$) for 11 weeks. It was found that feeding high levels of manganese retarded the regeneration of hemoglobin (see Fig. 4). The lambs on the manganese supplemented diets also had lower serum iron concentrations than those on the unsupplemented basal diet (see Fig. 5). The authors concluded that manganese interferes with iron absorption. They postulated that excessive manganese antagonizes the enzyme systems which oxidize or reduce iron at the site of absorption.

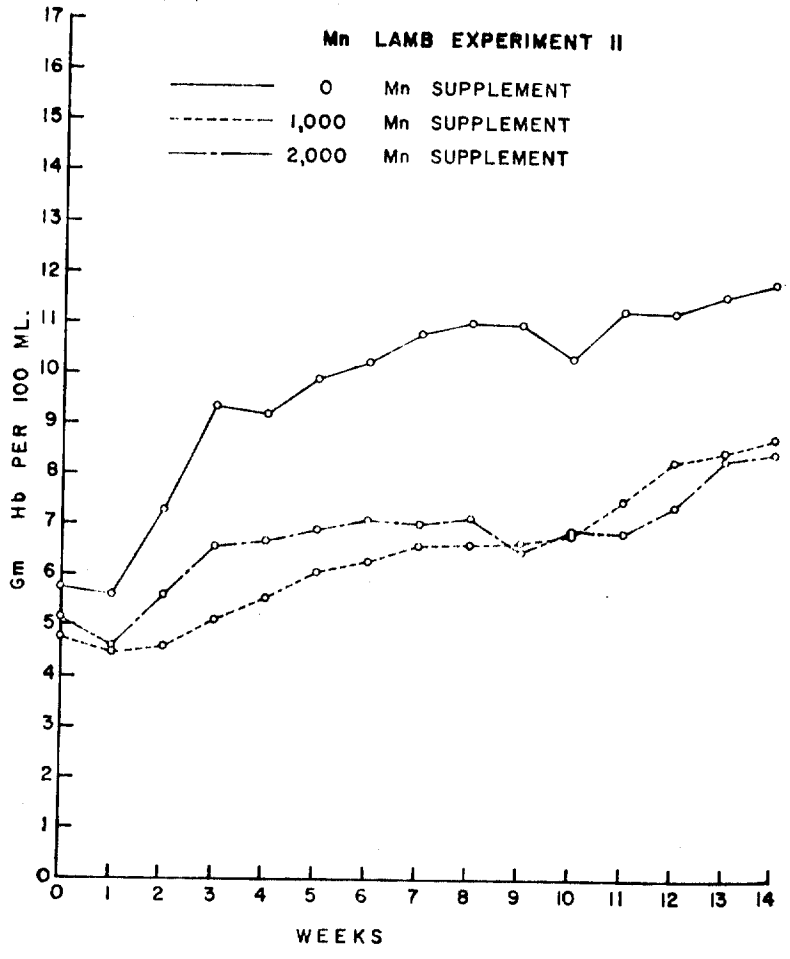


Fig. 4. Mean values of hemoglobin of the lambs fed three levels of manganese. (096)

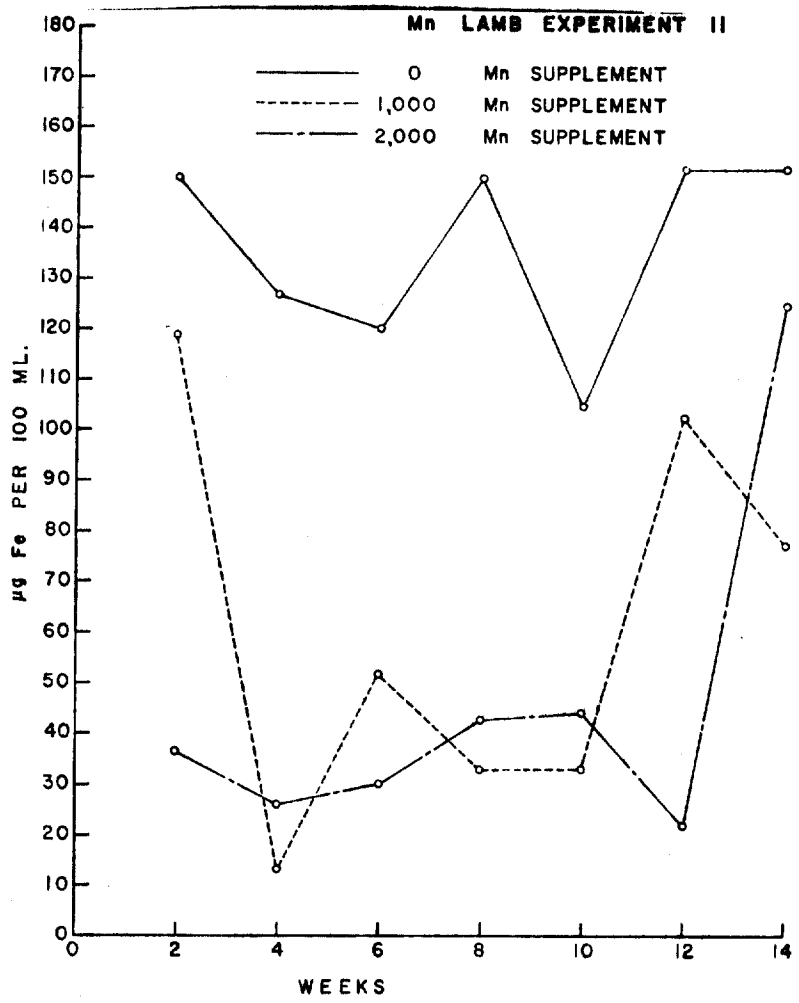


Fig. 5. Mean values of serum iron of the lambs fed three levels of manganese. (096)

H. Baby Pigs

Matrone et al. (158) determined the effect of excessive manganese (as $MnSO_4$) and additional iron in the diet on hemoglobin regeneration and also the minimal level of dietary manganese which retards hemoglobin regeneration. Anemia was produced in 12 new-born baby pigs. Then they were divided into groups of four and fed one of three diets (see Fig. 6) for 21 days. As can be seen on the figure, hemoglobin

was depressed with diets containing 2000 ppm Mn (as $MnSO_4 \cdot H_2O$). However, the manganese effect was overcome by the addition of 400 ppm iron.

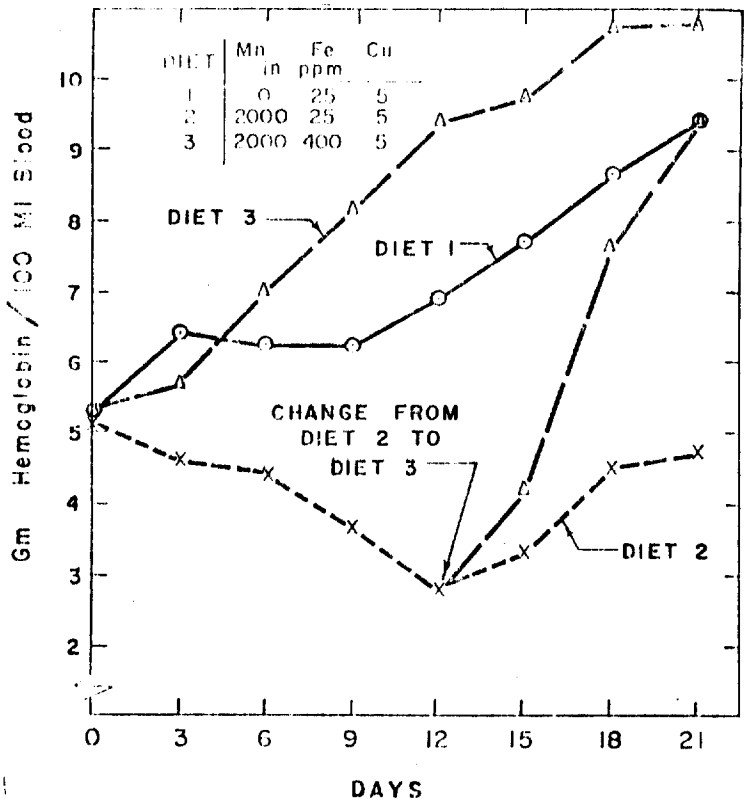


Fig. 6. Experiment 2, change in mean values of hemoglobin of baby pigs fed two levels of iron and two levels of manganese. (158)

In two other experiments, four levels of manganese supplemented diets were fed for 27 days; 0, 12, 250 and 2000 ppm. The weight gains and change in hemoglobin are shown in Table 18.

It was found that the largest weight gains were made by the pigs on the unsupplemented diet (control level) and the smallest by the pigs on the 2000 ppm supplemented diet. The hemoglobin increases for those pigs on 25 and 500 ppm were significantly lower ($P \leq 0.05$) than

for those on the unsupplemented diet. The smallest hemoglobin increment was made by the pigs on the manganese dietary supplement of 2000 ppm.

In a fourth experiment with anemic baby pigs, the levels of supplementary manganese studied were 0, 50, 250 and 1250 ppm. (See Table 18). It was found that the pigs on the unsupplemented diet gained the most weight and those on the 1250 ppm supplement gained the least. The minimum level of dietary manganese which interfered with hemoglobin formation was estimated to be between 50 and 125 ppm.

Table 18

Changes in Weight and Hemoglobin of Baby Pigs Fed Various Levels of Manganese[1] (158)

DIET NO.	LEVEL OF Mn	AV. GAIN PER PIG	AV. Hb INCREASE PER 100 ML BLOOD
	p.p.m.	*lbs.*	*gm*
	Experiment 3[2]		
1	0	28.8	6.09
4	125	26.2	3.38
5	500	26.0	3.18
2	2000	22.7	1.10
L.S.D.[3]		6.04	1.52
	Experiment 4[4,5]		
1	0	31.3	5.05
6	50	29.3	3.76
7	250	30.9	3.06
8	1250	27.8	3.24
L.S.D.		3.15	1.50

[1] Basal diet was made up of fresh cow's milk plus 25 p.p.m. of Fe and 5 p.p.m. of Cu and vitamins A and D.

[2] Three pigs per diet and an experimental period of 27 days.

[3] Least difference between any two means for significance at 5% level ($P = 0.05$).

[4] Six pigs per diet and an experimental period of 25 days for first three replications and 50 days for last three replications.

[5] After 25 days on experiment, iron of pigs on diets of last three replications in experiment 4 was increased from 25 to 50 p.p.m. (see text).

Underwood (262) considered that these findings suggest that the Mn:Fe dietary ratio is of wider significance than generally has been credited particularly because most livestock diets contain manganese in concentrations as high or higher than the ones cited in this experiment.

I. Monkeys

1. Neff (179) administered manganese dioxide (MnO_2) to monkeys. One ml of a suspension of MnO_2 in olive oil (200 mg/ml) was administered by subcutaneous injection to 15 squirrel monkeys (Saimiri sciurea). Controls (five) were injected with 1 ml olive oil. Injections were given twice, one month apart.

Two weeks following the first injection, four of the experimental and one of the control animals died. Some of the toxic symptoms developed by five of the remaining monkeys were: signs of muscular rigidity, flexion posturing of the extremities, and fine, rapid tremors of the distal extremities.

The remaining six test monkeys (and two controls) were given a third injection. This group of monkeys did not show uniformity in their toxic reactions: two appeared normal, three showed slight hand tremor, and one showed pronounced hand tremor along with impairment in equilibrium and coordination. (Effects observed on the brain biogenic amine concentration are discussed in the Biochemical Section).

2. Mella (166) administered manganese chloride over a period of 18 months to monkeys in an attempt to develop a syndrome similar to that produced by parkinsonism. Over the 18-month experimental period, four mature monkeys (Macasus rhesus) were intraperitoneally injected

on alternate days with gradually increasing doses of a sterile solution of 1 mg manganese chloride in 1 cc water. Two animals were kept as controls.

The symptoms typically showed by the monkeys were: first, development of choreo-athetoid type movements; next, rigidity accompanied by disturbances of motility; then, fine hand tremors; and finally, contracture of the hands with the terminal phalanges extended.

Histological examination typically showed:

a. In the brain, the patamen and caudate cells were shrunken and pyknotic; many had lost their nuclei, and there was occasional neuronophagia. The pallidal cells were large, reduced in number, swollen, vacuolated and showed eccentric nuclei; the large pallidal cells of the globus pallidus were shrunken and pyknotic, with some swollen and showing chromatolysis while others had eccentric nuclei and vacuoles.

b. Two livers showed acute hepatitis, i.e. areas of necrosis with small hemorrhages and fibrosis.

c. The manganese content of the brain was ten times the normal amount and that of the liver, about 15 times normal.

The author concluded that the observed disturbances of locomotion simulated those of humans which at the time of the paper (1924) were thought to be disturbances of the basal ganglions. The author also considered the fact that only the liver and brain showed any marked abnormality to be of significance.

3. Pentschew et al. (200) reported on the effects of repeated intramuscular injections of manganese dioxide. Five mature rhesus

monkeys were intramuscularly injected with manganese dioxide suspended in olive oil. All the monkeys developed similar neurological symptoms during the 9 to 24 month period after the start of the experiment. One animal was sacrificed 14-1/2 months after being given first 2000 mg MnO_2 and then three months later 3500 mg MnO_2. Another animal (no dosages given) was sacrificed 24 months after the beginning of the experiment.

The observed neurological disturbances in all the monkeys appeared to suggest dysfunction of the extrapyramidal motor system. The first monkey sacrificed showed spectacular alterations in the brain which were identical with those seen in human cases of manganese encephalopathy. The second animal sacrificed showed similar but more advanced alterations in the brain (see original paper for detailed discussion of the brain histology).

The authors concluded that the salient feature of manganese encephalopathy in man and primates appeared to be the severe, selective damage to the subthalamic nucleus and pallidum.

J. Humans

Kawamura et al. (121) reported an epidemic of manganese poisoning from drinking well water high in manganese. The poisoning was found to be due to large quantities of manganese dissolved in the well water as a result of 300 old dry cells buried in the well's vicinity. There were 16 people affected; five cases were severe, two were moderate and nine were mild. Two of the severe cases died, and one depressed moderate case committed suicide.

The symptoms, which resembled those associated with a motor

disturbance of the extrapyramidal tract, started with lethargy and edema. Autopsy showed atrophy and disappearance of the nerve cells of the globus pallidus. The authors pointed out that poisoning from dissolved, ingested manganese is relatively rare compared to that produced from inhalation. Until this study, previous reports were largely of isolated cases, 187 cases in all up to 1939.

III. Long-Term Toxicity

Rats

Becker and McCollum (012) kept rats on diets with and without high manganese supplementation for about two years. Young rats (40-50 g, number and strain not given) were fed a basal diet supplemented with 3.6% $MnCl_2 \cdot 4H_2O$, (0.9980 g manganese) for 730 days. The sexual function of males on the supplemented diet was apparently retained longer than in rats on similar unsupplemented diets. (The authors noted that since this long-term study was carried out with a small number of rats, they were checking it with a larger group of rats. This study was apparently never published since it was not found in the literature.)

IV. Special Studies

A. In Vitro

1. Krueger and West (131) performed systematic experiments on the effect of the electrolytes manganese chloride ($MnCl_2$) and manganese sulfate ($MnSO_4$) on the phage-bacterium reaction. It was observed that both these manganese salts in very low concentrations lessened the time for lysis to begin in a mixture of phage and bacterium (antistaphylococcus phage and a strain of Staphylococcus aurcus). Experimental results showed that for any given phage concentration the manganese-containing cultures lysed 0.5 hour ahead of the controls (see Table 19).

Table 19

Acceleration of Phage Action by Mn^{++} (131)

Initial phage concentration.....	1×10^8 P.U./ml.	1×10^7 P.U./ml.	1×10^6 P.U./ml.	1×10^5 P.U./ml.
Initial bacterial concentration.....	2.5×10^7 *B*/ml.	2.5×10^7 *B*/ml.	2.5×10^7 *B*/ml.	2.5×10^7 *B*/ml.
Time of onset of lysis: with Mn^{++}....	0.8	1.5	2.15	2.9
Time of onset of lysis: without Mn^{++}..	1.3	2.0	2.6	3.4

Temperature = 36°C. pH = 7.2.
For the Mn^{++} series the bacteria were grown in broth containing 0.00016 M $MnCl_2$.
The phage dilutions used contained the same concentration of $MnCl_2$.

Further experimentation showed that Mn^{2+} ion reduced the ratio of phage/bacterium required for lysis from 54 to about 12. Since the same effect was observed with both $MnCl_2$ and $MnSO_4$ solutions, the authors attributed it to the Mn^{2+} ion. They also found that in the presence of Mn^{2+} the distribution of phage is altered so that in growing phage-bacteria mixtures the extracellular phage concentration is increased four-fold. It was concluded that the accelerating effect of

that $MnCl_2$ affects the metabolic activity of bacterial cells in a way that makes the gene system more unstable.

3. Steinman et al. (249) analysed the interaction between the mutagenic compound, $MnCl_2$, and selected substances active in the mutational process. It was found that when applied to bacterial colonies for a few hours at 0.04% concentration, the mutagenic properties of $MnCl_2$ were appreciably affected by some, but not by other, mutagens. The mutational system employed was the reversal from streptomycin dependence to independence, and from cysteine requirement to prototrophy in strain Sd-4-73 of E. coli. The compounds tested are listed in Tables 20 and 21, and the experimental results are summarized in these two tables and Table 22. The concentrations used were as close to the toxicity level as the solubility of the compound allowed.

Table 20

Effect of a Variety of Agents on the Number of Mutants Induced by Manganous Chloride in E. coli Sd-4-73 at the Streptomycin-Dependence and Cysteine-Requirement Loci (249)

Test compound					Manganous chloride (0.04%)			Test compound plus 0.04% manganous chloride		
Name	Concn.	Survivors	Number of induced mutants per 10^8 survivors		Survivors	Number of induced mutants per 10^8 survivors		Survivors	Number of induced mutants per 10^8 survivors	
			sd-4	*cys-2*		*sd-4*	*cys-2*		*sd-4*	*cys-2*
	μg./ml.	%			%			%		
Actinomycin	SEE Table II									
Aminopterin	10	100	0	0	87.6	8200	80	93.8	9200	80
Amethopterin	5	100	0	0	41.4	3900	550	50.4	2100	620
8-Azaguanine	100	100	0	0	7.6	2200	180	100	2200	210
Azaserine	SEE Table III									
Benzimidazole	100	15.3	0	0	20.2	7400	290	33.8	2200	110
Carzinophilin	0.05	22.9	45	0	41.4	3900	550	<0.05	—	—
	0.01	47.2	310	0	100	210	31	26.7	2600	0
6-Chloropurine	10	60.2	1.6	0	78.1	800	70	70.1	500	50
Cycloserine	50	—	—	—	100	1100	120	32.3	1000	610
2,6-Diaminopurine	100	63.0	0	0	76.2	2200	80	63.1	1800	110
2,4-Diamino-5-*p*-chlorophenol-6-ethylpyrimidine (Daraprim)	10	92.3	0	0	94.2	1700	200	78.8	2200	260
2,4-Diamino-5-(3′,4′-dichlorophenyl)6-ethylpyrimidine	10	56.1	5	0	78.1	800	80	45.2	1600	150
6-Diazo-5-oxynorleucine (DON)	1000	68.0	0	0	55.2	2200	230	38.3	800	70
Kinetin	5	187.0	0	0	41.4	3900	550	35.1	3000	1200
6-Mercaptopurine	100	47.8	0	0	76.2	2200	80	50.6	5500	450
1,4-Dimethane sulfonoxybutane (Myleran)	10	100	0	0	94.1	1700	200	90.3	1600	170
Nutrient broth (Difco)	800	100	0	0	78.1	4100	250	76.2	2500	190
Puromycin	10	97.1	0	0	60.1	1200	380	42.4	1900	400
Triethylene melamine	5	100	970	2	41.4	3900	550	73.8	1400	190
Urethan	1000	93.8	0	0	87.6	1200	80	60.1	2700	230

Table 22

Combined Mutagenic Effect of Azaserine and $MnCl_2$ (249)

Expt.	Mutagens	Concn.	Survivors	Number of mutants per 10^8 survivors		Factor of synergism[a]	
				sd-1	*cys-2*	*sd-1*	*cys-2*
		μg./ml.	%				
1	Azaserine	5	35.3	1160	130		
	$MnCl_2$	400	87.6	8200	85		
	Azaserine + $MnCl_2$	5 + 400	1.4	520000	650	54	30
2	Azaserine	5	45.5	2600	40		
	$MnCl_2$	400	100	800	21		
	Azaserine + $MnCl_2$	5 + 400	11.5	7700	165	2.3	27
3	Azaserine	5	80.3	1700	260		
	$MnCl_2$	400	80.2	1100	180		
	Azaserine + $MnCl_2$	5 + 400	16.7	10400	1800	3.7	8.7
4	Azaserine	10	34.1	1400	14		
	$MnCl_2$	400	30.1	1000	16		
	Azaserine + $MnCl_2$	10 + 400	1.9	9500	120	4.0	1.9
	Azaserine + $MnCl_2$ + actinomycin	10 + 400 + 1	52.1	1500	12	0.6	0.4
5	Azaserine	1	42.4	1200	15		
	$MnCl_2$	400	100	3000	37		
	Azaserine + $MnCl_2$	1 + 400	67.2	170000	220	40.5	12

[a] The ratio of the experimentally assessed mutagenicity of the combination to the calculated sum of the mutagenic effects of the two separate components.

The significant observations were:

a. The interactions between $MnCl_2$ and other mutagens as measured by the two selected mutation rate changes were found to vary from highly synergistic to antagonistic (see Tables 20 and 21).

b. The combined action of $MnCl_2$ and azaserine exceeded the theoretical additive effect by a factor of 50 (see Table 22).

c. A few nonmutagens influenced the $MnCl_2$ activity as pro-mutagens or antimutagens (see Tables 20 and 21), for example actinomycin D consistently almost completely suppressed $MnCl_2$

mutagenic activity.

4. Sarachek (220) reported the extent to which the ability of manganous ions (Mn^{2+}) to induce the transformation to respiratory deficiency in non-dividing cells is dependent on the levels of the free amino acid pools of the cells and the magnesium ion concentration of the solvent medium in which they are exposed. A respiratory-sufficient, adenine-requiring stock of tetraploid Saccharomyces was the test organism used. For details of the methods used to deplete cells of their free amino acids and induce variants, see the original paper.

The results showed that in general the ability of Mn^{2+} (as $MnSO_4$) to induce respiratory variants and to inactivate cells is inversely proportional to the concentration of Mg^{2+} (as $MgSO_4 \cdot 7H_2O$) in the medium and directly proportional to the free amino acid levels of the cells (see Table 23). Under certain circumstances, for example by proper adjustment of Mg^{2+} and Mn^{2+} concentrations, the mutagenic properties of Mn^{2+} can be dissociated from its toxic properties.

Table 23

The Frequencies of Cell Survivals and of Respiratory Variants Appearing in Amino-Acid-Depleted and -Replenished Populations of *Saccharomyces* Exposed to $4 \cdot 10^{-3}$ M Mn^{++} and Various Concentrations of Mg^{++} (220)

Mg^{++} M	Alterations of the cellular free amino acid pool	Period of exposure to $4 \cdot 10^{-3}$ M Mn^{++}					
		24 h			72 h		
		% Survival	% Stable variants	% Total* variants	% Survival	% Stable variants	% Total* variants
$2 \cdot 10^{-3}$	undepleted	210	0.3	0.3	190	0.8	0.8
	depleted 5 h	100	0.2	0.2	95	0.3	0.3
	depleted 12 h	94	0.3	0.3	90	0.3	0.4
	replenished	109	0.2	0.4	98	0.4	0.9
$1 \cdot 10^{-3}$	undepleted	110	0.4	0.4	54	1.0	2.9
	depleted 5 h	97	0.3	0.3	88	0.3	0.4
	depleted 12 h	95	0.3	0.3	89	0.4	0.4
	replenished	100	0.5	0.6	48	0.6	1.0
$5 \cdot 10^{-4}$	undepleted	103	9.0	20.0	49	18.9	37.5
	depleted 5 h	88	0.3	0.6	62	1.4	2.6
	depleted 12 h	92	0.3	0.5	70	0.7	1.7
	replenished	90	2.8	3.6	12	6.8	9.6
$2.5 \cdot 10^{-4}$	undepleted	34	12.0	32.1	0.5	44.1	62.8
	depleted 5 h	70	1.8	3.5	29	38.4	47.6
	depleted 12 h	77	0.6	1.7	67	1.0	3.0
	replenished	50	5.2	10.1	18	30.0	43.0
$1 \cdot 10^{-4}$	undepleted	4.6	44.7	59.3	0		
	depleted 5 h	50	11.1	30.6	22	49.2	70.1
	depleted 12 h	67	1.6	5.3	30	3.2	12.3
	replenished	12	29.5	39.8	8	50.2	60.9

* Pooled values for stable and unstable respiratory variants.

The author concluded that heritable respiratory deficiency in non-dividing populations of Saccharomyces under the influence of Mn^{2+}, is the result of the decay of one or more essential physiological systems due to abnormal protein syntheses (or contingent RNA syntheses).

B. Mice

Popoff (204) demonstrated the effect which certain chemicals with a cell-stimulating effect have on the growth of mouse tumors. A solution was prepared consisting of the following components: 0.5 g $MgCl_2$ plus 200 mg $MnSO_4$, both these chemicals having previously been

identified as having a cell-stimulating effect; 2 cc glycerine; 15 drops calcium glycerphosphoricum; 3 drops tincture of iodine; 3 cc each of a 5% solution of LiCl and LiI, all dissolved in 40 cc "clear-filtered soil decoction".

The first experimental series involved 300 white mice in groups of 15-20 mice each with transplanted tumors derived from the same tumor. The experimental mice were given 8-10 subcutaneous injections of 0.5 cc test solution on the side of the body opposite the tumor, daily for two or three consecutive days with one rest day. There was one control group. It was observed that the tumors of the treated mice were one-half to one-third the size of the controls. The inhibitory effect on tumor growth only lasted for the duration of the injections. Tumor growth recommenced when the injections stopped. Over too long a period of injection, the solution had a deleterious effect.

In a second experimental series, 200 mice divided into groups of 15-20 mice and similarly transplanted with tumors derived from one initial tumor were fed a diet supplemented with 1 cc LiCl solution and 0.3 cc 10% $MnSO_4$ to which one drop of tincture of iodine was added every third day. The experimental mice showed a marked inhibition in tumor growth. Tumors were one-third to one-fourth the size of controls. As with the injected solution, continued administration had a deleterious effect. Also similarly, following cessation of treatment, tumor growth recommenced.

C. Rats

1. Elwood (064) investigated whether injected manganese chloride

caused histogenic and organogenic alterations in the oral cavity of rats. Subcutaneous injections of 1 cc of an aqueous solution of $MnCl_2$ (400 mg/kg BW) were administered to 20 rats (strain, age and sex not given). It was found that amelogenic disturbances and enamel defects were evident either histologically or grossly in the incisors of 100% of the treated rats. The clinically observed hypoplastic enamel lesions appeared between the 35th and 50th day post injection. No morphogenic alterations occurred. The results of the same experiment with hamsters and guinea pigs are given later in this section.

2. Mackiewicz (152) investigated whether manganese has an effect on anemia in rats. Anemia was first induced in 65 young rats by three methods: (1) the method of Elvehjem and Kemmerer (23 animals) in which weaned rats were fed only vitamin A and D supplemented cow's milk for three weeks; (2) the modified Elvehjem-Kemmerer method (18 animals) in which in addition to milk the animals received normal food for nine days; and (3) bleeding from the tail vein (24 two-month old rats).

A 0.25% $MnCl_2$ solution was subcutaneously injected in doses of 5 mg/kg BW (1.3 mg/kg manganese) for four weeks. In the first experimental group, 15 animals were injected and eight were controls. In the second group, 12 were injected with six as controls and in the third group, 12 were injected and 12 were controls. Determinations were made on bone marrow and peripheral blood which included hemoglobin levels, red cell counts and reticulocyte counts. It was found that there was no difference in the behavior of these components between experimental and control rats. The author concluded that irrespective

of the method of anemia induction, manganous chloride had no effect on the course of the anemia.

D. Hamsters

Elwood (064) investigated whether $MnCl_2$ causes histogenic and organogenic alterations in the oral cavity of hamsters. Twenty hamsters were each given a subcutaneous injection of $MnCl_2$ in doses ranging from 150-550 mg/kg BW. Three untreated animals and two injected with sodium chloride were the controls. No hypoplastic enamel lesions developed in either the experimental or control animals.

E. Guinea Pigs

Elwood (064) also investigated whether $MnCl_2$ causes histogenic and organogenic alterations in the oral cavity of guinea pigs. Two experimental groups were observed:

(1) Twelve animals were subcutaneously injected with a 1 cc aqueous solution of $MnCl_2$ in doses ranging from 150-490 mg/kg BW. Two controls were each injected with 1 cc of a sodium chloride solution containing chloride equivalent to 300 mg/kg BW $MnCl_2$. Two other controls were not injected.

(2) Eight animals were subcutaneously injected with doses of sodium chloride containing chloride equivalents ranging from 400-700 mg/kg BW $MnCl_2$. Two controls were untreated.

Hypoplastic enamel defects were observed in the incisors of 100% of the surviving animals between the 56th and 73th days post injection. All the incisors also showed a dual grooving which is a manifestation of a disturbance of morphogenesis. The hypoplastic

pitting in each case developed in relation to the grooves. The sodium chloride injected guinea pigs did not develop these incisor defects.

F. Chicks

Gallup and Norris (080) attempted to determine the function of manganese in preventing perosis, by using a variety of manganese salts. One experiment was carried out with day-old New Hampshire chicks in groups of about 25 each which were fed a basal diet to which one of the following salts was added to supply 50 ppm manganese in the diet: $MnCl_2 \cdot 4H_2O$; $MnSO_4 \cdot 4H_2O$; $KMnO_4$; $MnCO_3$; and MnO_2.

In a second experiment with seven groups of 25 day-old chicks, manganese carbonate ($MnCO_3$) was added to the basal diet in amounts increasing from 20 to 1000 ppm of diet.

The results of these two experiments, summarized in Tables 24 and 25, showed that when these manganese salts supply manganese at the rate of 50 ppm of diet, they are effective in preventing perosis in chickens.

A third experiment supplied manganese to other groups of chicks by means of $MnCl_2$ and potassium permanganate ($KMnO_4$) dissolved in equivalent amounts in the drinking water. The results of this experiment are summarized in Table 26. They showed that additional amounts of manganese dissolved in the drinking water were ineffective in prevention of perosis which developed early.

These experiments also showed that manganese deficiency prevents optimum growth of chicks and pullets, and that large quantities of manganese (1000 ppm of diet) were not toxic.

Table 24

Perosis-Preventive Properties of Different Manganese Salts (080)

Group	Chicks per group	Addition to basal diet	Manganese in diet	Av. wt. of chicks at 6 weeks	Cases of perosis	Severity of perosis
	no.		p.p.m.	gm.	per cent	
1	27	None	10	383	81.5	38.8
2	25	$MnCl_2.4H_2O$	50	547	4.0	2.0
3	23	$MnSO_4.4H_2O$	50	591	13.0	6.0
4	25	$KMnO_4$	50	558	0.0	0.0
5	25	$MnCO_3$	50	557	4.0	2.6
6	24	MnO_2, 85%	50	560	4.1	1.8
7	25	$MnSO_4.4H_2O$	100	574	4.0	2.1

Table 25

Amount of Manganese Required to Prevent Perosis in Chicks on Diet 3.000 (080)

Group	Chicks per group	Manganese[1] content of diet	Av. wt. of chicks at 6 weeks	Cases of perosis	Severity of perosis
	no.	p.p.m.	gm.	per cent	
1	23	10	386	74.0	36.6
2	25	20	523	56.0	21.5
3	25	30	518	16.0	8.0
4	26	40	536	11.0	2.8
5	25	50	535	4.0	2.0
6	24	70	547	8.2	3.7
7	25	500	538	4.0	1.5
8	24	1,000	541	4.1	2.0

[1] Manganese provided by means of $MnCO_3$.

Table 26

Perosis-Preventive Value of Manganese When Supplied in the Drinking Water (080)

Group	Manganese content of diet	Manganese content of water	Average daily manganese intake during—		Av. wt. of chicks at 6 weeks	Cases of perosis	Severity of perosis
			1st week	2nd week			
	p.p.m.	mg. per ml.	mg.	mg.	gm.	per cent	
12	10	0	0.08	0.16	538	74	32.4
13	40	0	0.43	0.76	606	4	0.4
17	10	0.15[1]	3.86	5.81	582	4	1.8
18	10	0.015[2]	0.30	0.70	606	4	1.3
19	10	0.015[3]	0.35	0.68	556	4	2.0

[1] Manganese supplied in the water as $MnCl_2$ during the first 2 weeks only. Thereafter the chicks received manganese in the diet, 40 p.p.m. as $MnCO_3$.
[2] Manganese supplied by $KMnO_4$.
[3] Manganese supplied by $MnCl_2$.

G. Rabbits

1. Walbum and Schmidt (275) found that $MnCl_2$ was the most effective metal salt (of those tested) in the formation of amboreceptors in rabbits. This experiment was carried out as part of a continuing study of the effect of metal salts on the immunologic process. (See the original paper for details of the experimental procedure.) The effect of the injected metal salt was determined by the level to which it was able to raise the amboreceptor concentration in the serum relative to the maximum amboreceptor concentration obtained with antigen alone. The results for $MnCl_2$, using three rabbits were:

	First Peak	Amboreceptor units per cc before the salt injec.	second peak	Percent increase	Average increase
$MnCl_2$	1920	833	1470	33	28
	1470	1000	1470	32	
	1430	714	1000	20	

2. Dechigi and Torrelli (055) observed the immunological effect of progressive $MnCl_2$ injections in rabbits. A series of tests were carried out. In the first experiment, five rabbits were vaccinated with Vibrio cholerae. Bacteriolysis and agglutination tests were done six days after the last injection of vaccine. Then three rabbits (two were kept as controls) were intravenously injected with 1 cc n/100 $MnCl_2$ solution (0.63 mg $MnCl_2$ in 1 cc). The quantity of agglutinin and lysin were considerably increased after five hours while they remained relatively stable in the controls.

After a five-day period the three experimental rabbits were intravenously injected with 1 cc n/10 $MnCl_2$ solution (6.3 mg $MnCl_2$ in 1 cc). This concentration also produced an increase in antibody production.

After another five days the experimental animals were intravenously injected with 1 cc standard $MnCl_2$ solution (63 mg $MnCl_2$ in 1 cc). This concentration produced only a slight increase in immunizing capacity.

After a final five-day period the test animals were intravenously

injected with 1 cc 1:10 $MnCl_2$ solution (100 mg $MnCl_2$ in 1 cc). A considerable decrease in the agglutinin and lysin rate was observed (these rates remained nearly constant for the controls).

The authors concluded from the data (see original paper for tables and diagrams) that small and average $MnCl_2$ doses had a strong stimulating effect on the production of the two antibodies studied, whereas large $MnCl_2$ doses resulted in a marked but temporary decrease in their rate of production. The antibody rate increased again after a period of time. The authors attributed this temporary decrease in antibody production to the momentary high level of manganese which exerts an intoxicating effect thus resulting in an inhibitory phase of production of these particular antibodies.

In a second series of experiments the authors investigated what effect repeatedly administered $MnCl_2$ would have on the immunity state. Eleven gray rabbits (1800 g) were divided into three groups:

a. Five rabbits intravenously injected with both small and large doses of $MnCl_2$ before vaccination.

b. Four rabbits intravenously injected with doses of n/100 $MnCl_2$ solution (0.63 mg $MnCl_2$/cc) after vaccination.

c. Two controls.

(See the original paper for experimental details and tables.) The observations made were:

a. The increase of the antibody rate in animals first intoxicated with $MnCl_2$, and then vaccinated was moderate and, in general, to the same extent as the controls.

b. The rabbits in the group that was first vaccinated and then

intoxicated with $MnCl_2$ formed a large quantity of antibodies (particularly the agglutinins) as compared to controls. This was followed by a decrease which was comparable to the decrease in the controls.

The authors concluded from these observations that: (1) Preventive poisoning with manganese "does not leave indelible traces" in all organic systems involved in agglutinin production, provided that agglutinin production is normal. (2) In animals first vaccinated and subsequently intoxicated with $MnCl_2$, the intoxication favors the already established production of antibodies.

H. Cats

Belokon (013) investigated the effect of manganese (as $MnCl_2$) on the electrical activity and reflex excitability of the spinal cord of cats. The experiments were carried out on 52 anesthetized cats intravenously injected (femoral vein) with $MnCl_2$ solution (0.0001-0.01M; 0.01-1 mg/kgBW Mn). The solution was also locally applied to the place where potentials were detected.

Following $MnCl_2$ intravenous injection there was an initial increase in the background electrical activity of the spinal cord. An increase in the amplitude of the mono- and polysnaptic reflex responses, followed by a return to their initial level was also observed. No similar appreciable changes were noted in a series of control experiments in which 0.9% sodium chloride solution was intravenously injected.

The author concluded that:

a. The changes in the electrical responses of the spinal cord apparently are principally dependent on the direct influence of

manganese salts on its functional state. Presumably, following the manganese injection there is a stimulation of redox processes.

b. The observation that the amplitude of the polysynaptic reflexes is increased much more than that of the monosynaptic reflexes indicates that manganese ions have a greater influence on the interneurons than on the motoneurons of the spinal cord.

I. Dogs

Conrad et al. (042) investigated the acute effects of manganese on cardiac contractility, heart rate, and blood pressure in dogs subjected to thoracotomy, under a variety of experimental conditions. These included: beta adrenergic blockade, ablation of pressor reflexes by total spinal anesthesia and vagotomy, adrenalectomy, and pretreatment with reserpine. Manganese chloride in doses of 25-100 mg in 1 ml distilled water was intravenously injected into 17 of the dogs. Two control animals were injected with 1 ml of a solution of sodium chloride (4.6%) equivalent in tonicity to a solution containing 100 mg $MnCl_2$.

It was observed that except in animals subjected to either beta adrenergic blockade with pronethalol or bilateral adrenalectomy, an increase in myocardial contractile force resulted from every $MnCl_2$ injection. The overall magnitude of this positive inotropic effect averaged 155% of the control values. During the peak inotropic effect an increase in heart rate and widening of the pulse pressure were observed.

The authors concluded that the mechanism by which manganese produces a positive inotropic effect is indirect. It takes place by

indirectly stimulating the adrenal medulla with subsequent release of catecholamines which increase cardiac contractility as well as restore the blood pressure to normal.

J. Humans

1. Normet (183) summarized his observations concerning the therapeutic action of manganese citrate. He prepared a formula containing manganese citrate, magnesium citrate, ferric-potassium tartrate, water and glycerine.

Some of the findings were:

a. When the manganese citrate content of the formula was 0.40 g/1000 g to 1 g/1000 g an intravenous injection of 0.25 cc/kg BW, caused elevated temperature, chills and profuse sweating. Six to seven hours following injection the temperature returned to normal. There was no reaction when the manganese citrate concentration was below 0.20 g/100 g.

b. There appeared to be an improvement in hemoglobin formation in anemia when the manganese citrate concentration was reduced to 0.05 g/1000 g (0.0001 g manganese) of the formula.

c. Positive results were obtained in the treatment of influenza, cerebrospinal meningitis, measles and icterohemoglobinuria. The treatment of typhoid fever was not effective. (No details were given for these diseases.)

d. Positive results were obtained with the formula in the treatment of a case of bronchopneumonic influenza (a case history was given).

2. Gorlitzer (084) reported the successful treatment of 17 cases

of "rheumatic" endocarditis and one case of acute tonsillitis with intravenous injections of a 0.02 molar solution of $MnCl_2$.

3. Mehrotra et al. (164) reported the results of a clinical trial in which orally administered $MnCl_2$ was tested as a hypoglycemic agent with unselected cases of diabetes mellitus. Fifteen diabetics (30 to 62 years old and one 14-year-old), six of whom were thin and nine heavy, were administered varying doses (5-250 mg) of $MnCl_2$ orally with 50g glucose.

It was found that:

a. There was a statistically significant fall in blood sugar levels, but the hyperglycemia could not be controlled with a single dose.

b. Increasing $MnCl_2$ doses did not produce a corresponding fall in the blood sugar levels.

c. There were no observed side-effects due to the $MnCl_2$.

d. Thin diabetics responded better than heavy diabetics.

The authors postulated that manganese chloride activates enzymes involved with the metabolism of carbohydrates but only up to a certain point. Beyond that point, increasing the dose does not correspondingly increase the hypoglycemic action.

4. Artamonova (006) reported on clinical tests with $MnCl_2$ in the treatment of nonspecific infectious polyarthritis. Intramuscular injections of a 1% aqueous solution of $MnCl_2$ were administered in doses of 0.1 ml to 1.5 or 2.0 ml alternate days for a total of 15 to 20 days to 30 patients (23 females and 7 males). No toxic side effects were observed.

Good or satisfactory results were obtained in 22 of the patients.

In ten, pain in the joints was completely relieved. In the other 12, the pain diminished considerably, edema in the vicinity of the joints subsided, and there was an increase in mobility of the joints. Laboratory indices of the inflammatory process did not show changes paralleling the clinical improvement.

The author concluded that since administration of manganous chloride mitigates the clinical course of nonspecific infectious polyarthritis, it is recommended for treatment of this condition.

BIOCHEMICAL ASPECTS

I. Breakdown

There is no specific information in the literature concerning the breakdown of manganese compounds in the body. However Cotzias (044) has pointed out that manganese is present in foods as coordination complexes. These coordination compounds act differently from inorganic manganese salts with respect to absorption in the gut and transport across cell walls. Von Oettingen (269) has noted that the differences in the degree of solubility among various manganese compounds are reflected in the level of manganese found in the bloodstream following administration. Thus the evidence would appear to indicate a wide variation in the breakdown of manganese compounds which depends on their source, whether they are inorganic salts or chelates, and their solubility in the gastric juice.

II. Absorption and Distribution

Introduction

Von Oettingen (269) concluded after reviewing several experimental observations that when manganese salts are administered in small amounts, they are only slowly and incompletely absorbed in the gut, resulting in only a temporary increase of the manganese level in the bloodstream. Large amounts are apparently absorbed better.

Dogan and Beritic (057) in their review noted that when evidence of absorption is not seen even with large amounts of certain manganese compounds, it is due to their poor solubility in the gastric juices. This is particularly true of the oxides. The authors cited an early experimental study with orally administered franklinite, rhodonite and manganese dioxide which showed their solubility to be dependent on the acidity of the stomach and

the duration of contact. Even though these compounds were at best only slightly soluble, it was sufficient for absorption of manganese into the bloodstream.

Cotzias (044) pointed out that there is not necessarily a parallel between the rate of absorption of large amounts of inorganic manganese salts and that of the trace amounts found in foods. The manganese in foods is largely present in coordination compounds. Since the rate of transport across any cell wall is a function of the ind vidual coordination complex, the differences in behavior between inorganic and coordinated manganese compounds would be significant.

Schroeder et al. (222) concluded from the knowledge contributed by the research of Cotzias and his coworkers that when divalent manganese is ingested as an ion or in chelates it probably remains unchanged in the acid stomach, but in the alkaline small intestine conditions are favorable for oxidation. The manganese which is absorbed in the general circulation is transported by transmanganin, a B_1 globulin in plasma, as the trivalent ion. The alkaline state of body fluids in mammals is favorable for retention of manganese in the trivalent state. The absorbed manganese after transport to the liver is conjugated with bile and excreted into the gut where part is reabsorbed in a small enterohepatic circulation.

A. In Vitro

Cikrt (038) studied the uptake of four metals including ^{52}Mn (as the chloride) in the duodenal and ileal segments of the small intestine in vitro by the modified method of everted intestinal sacs. Only the results with ^{52}Mn will be discussed here. Female Wistar rats (180-200 g) were used for the experiment.

^{52}Mn (as the chloride) at concentrations of 10^{-5} and 10^{-6}M was added to the mucosal medium to study the uptake by ileal and duodenal segments. The results showed that compared to the three other metals studied, ^{52}Mn showed: (a) the lowest decrease in the incubation medium during incubation; (b) the lowest uptake by the intestinal wall; (c) the highest values in the serosal medium; and (d) inhibition of uptake by the intestinal wall during anaerobic incubation (bubbling with nitrogen). The author concluded that these results support the assumption that there is an active transport of Mn through the intestinal wall, in vitro.

B. Mice

Britton and Cotzias (030) observed the effect of stable manganese intake on distribution in the tissue and elimination rate. A total of 180 Swiss albino mice (Hale-Stoner strain, 4-5 weeks old) were used in five experiments. After a variable period of prefeeding, the mice were fed diluted evaporated milk supplemented with manganese sulfate ($MnSO_4$) in concentrations ranging from 2.3×10^{-7} to 1.25×10^{-1}M (18 µg to 6.9 g Mn^{2+}/liter). (See Table 27 for details.) Standardized doses of 4 or 5 µc carrier-free $^{54}MnCl_2$ in 0.1 ml 0.9% NaCl solution were intraperitoneally injected.

Table 27

Concentrations of Mn in Diets of Various Groups (030)

Exp. No.	Group	Basal Diet, Days	Supplement (Molarity $MnSO_4 \cdot H_2O$)
1	1-3	5	4.69×10^{-7} M; 1×10^{-3} M; 2×10^{-3} M
2	1-4	8	4.69×10^{-7} M; 5.01×10^{-4} M; 4×10^{-3} M; 8×10^{-3} M
3	1-3	11	4.69×10^{-7} M; 1.6×10^{-3} M; 3.2×10^{-3} M
4	1-12	19	4.69×10^{-7} M; 5.01×10^{-4} M; 1×10^{-3} M; 2×10^{-3} M; 4×10^{-3} M; 8×10^{-3} M; 1.6×10^{-2} M; 2.4×10^{-2} M; 3.2×10^{-2} M; 4.8×10^{-2} M; 6.4×10^{-2} M; 1.25×10^{-1} M
5	1-12	26	4.69×10^{-7} M; 7.89×10^{-7} M; 2.07×10^{-6} M; 8.47×10^{-6} M; 4.05×10^{-5} M; 2.01×10^{-4} M; 1×10^{-3} M; 5×10^{-3} M; 2.5×10^{-2} M

Two separate types of experiment were carried out: (1) the different amounts of $MnSO_4$ were given immediately following a prefeeding period of from 5 to 26 days (experiments 1 to 4, Table 27); and (2) the basal low-manganese diet was continued for 16 days after the isotope injection, before the various $MnSO_4$ concentrations were added to the diets (experiment 5, Table 27).

The results of these experiments indicated that the isotope was excreted in proportion to the amount of metal supplied in the diet. A linear relationship was shown between the level of manganese in the diet and the excretion rate. Investigation of the ^{54}Mn distribution among various organs suggested to the authors that when excess manganese was fed, the metal was absorbed from the diet in proportion to the amount presented for absorption. From this it was concluded that the relative stability of manganese concentrations in the tissues is due to controlled excretion rather than to a regulated absorption.

These experiments also showed that the retained radioisotope became increasingly unavailable for exchange with stable Mn^{2+} as a function of time after administration. This indicated that the excretory routes responded rapidly to the administration of excess $MnSO_4$. In these experiments it was demonstrated that manganese turnover in the tissues is dependent on the supply.

C. Rabbits and Guinea Pigs

Lemos (140) studied the distribution of manganese following administration of different manganese compounds to rabbits by various routes. (For the details of the methods of organ analysis used, see the original paper.)

Thirteen rabbits and one guinea pig were used in the experiment; one rabbit served as a control. The results of these experiments are shown in Tables 28, 29 and 30.

From Table 28, it can be seen that:

a. Large oral doses of $MnCl_2$ were toxic and localized chiefly in the heart, kidneys and liver. A noteworthy observation is the high levels found in the suprarenals, bone marrow, bile and skin.

b. Manganese sulfate ($MnSO_4$) absorbed daily in moderate doses is not excessively absorbed.

c. Intramuscular injections of $MnSO_4$ result in high manganese levels in the suprarenals, bone marrow and spleen.

d. Injected permanganate, which was toxic, resulted in high manganese levels in the spleen, heart and suprarenals.

From Table 29, it can be seen that in the animals which absorbed manganese dioxide orally, the amount found in the principal organs,

e.g. the brain, increased in relation to the duration of the treatment. The bile, skin and hair also showed high concentrations related to their role as routes of elimination.

From Table 30, it can be seen that in animals exposed to inhalation of manganese dioxide dusts, there is a similar increase in manganese in the principal organs related to duration of exposure. The largest amounts were found in the heart, kidneys, lungs and brain. Significant amounts were also found in the suprarenals and the bone marrow.

The author concluded that the high levels frequently found in the small organs, e.g., the suprarenals, bone marrow, testicles and spleen, may, along with localization in nerve centers, explain accidents reported with workers exposed to prolonged absorption of significant quantities of manganese-containing substances.

Table 28

Manganese in Milligrams per 100 g Fresh Organ (140)

Organs sampled	Rabbit no. 1 Normal	2 $MnCl_2$ oral route	3 $MnSO_2$ oral route	4 $MnSO_4$ injected	5 $KMnO_4$ injected
Liver	0.08	6.3	0.31	11.12	0.02
Heart	0.03	61.9	0.46	4.75	5.1
Kidneys	0.01	9.5	0.45	3.58	0.47
Lungs	Traces	2.75	0.14	2.34	0.81
Brain	0.67	0.44	1.36	1.10	0.13
Spleen	Traces		Traces	4.2	2.9
Muscles	0.01	0.43	0.23		0.40
Blood	Traces		0.18	2.8	0.19
Skin and hair	Traces	3.60	0.30		0.28
Bile	0.30	10.8			
Testicles or ovaries	0.02	1.61	Traces	1.6	Traces
Suprarenals	Traces	50.6	Traces	5.0	10.9
Bone	Traces	0.46	Traces	0.96	Traces
Bone marrow		10.3		6.33	

Table 29

Manganese in Milligrams per 100 g Fresh Organ (140)

Organs Sampled	Rabbit No. 6	7	8	9	10
	MnO_2 orally			Oil suspension of MnO_2 by esophageal tube	
	2 mos.	3 mos.	5 mos.	3 doses	6 doses
Liver	3.92	0.94	0.95	1.26	1.47
Heart	0.27	1.87	2.5	3.8	4.38
Kidneys	1.00	1.35	1.90	1.1	4.99
Lungs	0.07	0.80	1.7		
Brain	1.07	3.01	5.8	0.66	1.26
Spleen	Traces	Traces	Traces	0.23	8.12
Muscles	0.54	0,47	0.45		0.38
Blood		0.25	0.26		0.59
Skin and hair	1.49	2.20	3.9		1.13
Bile	25.4		54		
Testicles	0.71	0.41	11.9		2.12
Suprarenals	Traces		18.8		Traces
Bone	0.95	0.34			0.20
Bone marrow	3.35				1.66

Table 30

Manganese in Milligrams per 100 g Fresh Organ (140)

Organs sampled	Rabbit No. 11	Rabbit No. 12	Rabbit No. 13	Guinea Pig 14
	Inhalation of MnO_2			Inhalation of MnO_2
	50 hrs.	125 hrs.	300 hrs.	210 hrs. and food containing MnO_2
Liver	0.45	1.11	1.5	2.2
Heart	1.00	2.84	3.06	3.1
Kidneys	0.71	1.35	3.04	3.4
Lungs	2.4	3.8	3.2	1.2
Brain	0.9	0.68	1.52	5
Spleen	1.05	Traces	Traces	4.0
Muscles	0.38	0.39	0.67	0.86
Testicles	Traces	Traces	2.5	1.3
Suprarenals . . .				7.0
Bone	1.2	0.78	1.7	4.0
Bone marrow . . .	0.64	1.3	10.8	

D. Cows

Anke et al. (003) investigated the comparable ability of the blood, the milk and the hair of cows to reflect the incorporation of ^{52}Mn after oral administration of one of the following isotopic manganese compounds: $^{52}MnSO_4$, $^{52}MnCl_2$ and $^{52}MnO_2$ (reduced). For 11 days preceding the experiment, 15 Holstein cows divided into three groups of five each were fed one of the manganese compounds along with a winter ration.

At the start of the experiment, ^{52}Mn (150 mg) as an aqueous solution of one of the compounds with an activity of 4.5 mc per cow was administerd by esophageal tube to five cows. Samples of black body hair, blood from the jugular vein and milk were taken 6, 12, 18, 24, 48 and 96 hours after administration of the particular radioisotopic manganese compound.

The results showed the following:

(1) There was no isotopic manganese (^{52}Mn) in the milk.

(2) The ^{52}Mn values in the blood increased continuously ($P<0.01$) until 96 hours after administration of the isotope (the end of the experiment). No significant differences were found among the groups receiving the three different compounds.

(3) The maximum values for ^{52}Mn activity retained in the black body hair were 10 to 18 times higher than those for blood. There was no difference found in the content of ^{52}Mn in the black body hair between cows receiving $^{52}MnSO_4$ or $^{52}MnCl_2$. The hair of cows receiving these two compounds however, contained significantly less ^{52}Mn than that of cows fed reduced $^{52}MnO_2$ ($P<0.011$, 0.05).

The authors concluded from these observations that: (1) the Mn content of milk and blood are not usable to indicate the Mn supply of cows, (2) since Mn is stored for a short time in the black body hair, the Mn content of hair can serve to indicate Mn supply, and (3) the three manganese compounds administered in this experiment are all utilizable as a Mn source for cows.

E. Monkeys

Mella (166) intraperitoneally injected manganese chloride (1 mg $MnCl_2$ in 1 cc water) on alternate days in gradually increasing doses over a period of 18 months to four mature monkeys (Macasus rhesus). The manganese content of the tissues examined is shown in Table 31. The manganese content of the brain was found to be ten times the normal value and that of the liver fifteen times normal. Both these organs showed marked pathological changes. (For details of this experiment see the Short Term Toxicity Section.

Table 31

Manganese Content of Tissues (166)

	Mg. Per 100 Gm. of Tissue
Liver	0.120
Lung	0.055
Brain	0.032
Kidney	0.155
Pancreas	trace
Spleen	0.100
Heart	0.070

F. Humans

Cotzias et al. (047) compared the rate of loss of injected ^{54}Mn from the whole body and from an area representing the liver between a normal population of "healthy" manganese miners and patients suffering from chronic manganese poisoning.

Three groups of subjects were used: (1) Normal men and women (9) between 20 and 30 years of age, as controls. (2) Healthy working miners (20) between 23 and 60 years of age. (3) Patients with chronic manganese poisoning (18) between 18 and 56 years of age. Single calibrated doses of 40 μc carrier-free ^{54}Mn in 0.9% sodium chloride solution were injected into an antecubital vein.

The observations made were:

(1) Most of the radioactive tracer was cleared from the bloodstream with a mean half-time of between 1.3 and 2.2 minutes. Of the three groups studied, the healthy miners showed the slowest clearance from the bloodstream.

(2) After the initial clearance phase, the remaining tracer behaved differently in whole blood than in plasma. There was an increase in the concentration of the tracer in whole blood, but it did not change in plasma over a 30-day observation from the half-time clearance level.

(3) When whole blood, serum, cerebrospinal fluid, urine, hair, skin, and muscle were examined for ^{55}Mn content, it was found that healthy manganese miners carried higher tissue concentrations of ^{55}Mn than did the group of former miners with chronic manganese poisoning.

The authors concluded that the elevated tissue concentrations are

related to exposure but are not necessary for the presence of neurological symptoms of chronic manganese poisoning. They made the assumption, therefore, that metal chelation therapy would not be effective in reversing these neurological manifestations.

III. Metabolism and Excretion

Introduction

In his review Cotzias (044) discussed the experimental evidence he and his coworkers had obtained which indicated that the manganese excreted with the bile into the intestine is partially reabsorbed and excreted again.

Schroeder (222) in his review points out that when the hepatic excretion route is blocked, the pancreas maintains homeostasis by serving as a reserve organ of excretion for absorbed manganese.

Underwood (262) in his review noted that experimental work with several species including man has shown that manganese is almost completely excreted via the intestinal wall by several routes. These are interdependent and provide the body with an efficient mechanism for regulation of the manganese concentrations in the tissues. This controlled excretion is responsible for the relative stability of manganese concentrations in the tissues. Underwood (262) states, as others have also pointed out, that the bile flow is the main route of excretion and is thus the chief regulatory mechanism. If there is a manganese overload or the biliary route is blocked, excretion also occurs via such auxiliary routes as the pancreatic juice, into the duodenum and jejunum and to a lesser extent into the terminal ileum. There is very little manganese excreted in the urine.

A. Mice

1. Cotzias and Greenough (046) studied the specificity of the manganese pathway through the mouse's body and observed the rate of elimination of radiomanganese from the body.

The experimental animals were male Swiss albino mice (7 weeks old, 16-18 g), intraperitoneally injected with 1.0 μc $^{54}MnCl_2$ in 0.2 ml saline. The injected radiomanganese was challenged by a variety of metals, metal salts, as well as manganese carriers in valence states of 0 (metal powder) to 7 (permanganate). (For details see Table 32.)

The significant observations made from these experiments were:

a. Challenge with stable manganese in any valence state promoted the rapid excretion of the body's radiomanganese.

b. None of the other stable metals tested (see Table 32) were able to affect in any way the animal's normal rate of radiomanganese elimination.

c. When manganous, ferrous or chromous citrates were injected, only the manganese compound had an appreciable effect on the distribution of the tracer among the animals' organs. The distribution after injection of ferrous or chromous citrates, even in concentrations 50 times greater than an effective amount of $MnSO_4$, was comparable to that of the controls.

These observations led the authors to conclude that there is a specific intracellular manganese pathway through the body, and that the irreplaceability of manganese in the body by other metals is evidence that manganese performs specific tasks which cannot be taken over by other metals.

Table 32

Summary of Metals Administered to Manganese^{54} Injected Mice (046)

No. of mice	Metal or salt	Atomic number	Moles per 20 Gm. mouse*	Moles per challenge	At. wt. isotope
7	$MgSO_4$	12	4.2×10^{-4}†	$5 \times 10^{-6} - 5 \times 10^{-6}$	146
3	$VOSO_4$	23	1.3×10^{-7}‡	1.0×10^{-6}	310-505§
9	Cr Cit	24	3.1×10^{-7}‡	$2 \times 10^{-6} - 1 \times 10^{-5}$	(−)6 505§
13	$CrCl_2$	24		$4 \times 10^{-6} - 1 \times 10^{-5}$	50 650
3	Mn^0	25	2.8×10^{-7}‡	$9 \times 10^{-6} - 4.5 \times 10^{-4}$	6-146
11	Mn Cit	25		$1.8 \times 10^{-6} - 9 \times 10^{-6}$	(−)6 674§
28	$MnSO_4$	25		$9 \times 10^{-6} - 1 \times 10^{-5}$	50 650
3	MnO_2	25		1.1×10^{-4}	(−)24
3	$KMnO_4$	25		2.5×10^{-6}	354
6	$FeCl_3$	26	2.1×10^{-6}‖	$9 \times 10^{-6} - 1 \times 10^{-5}$	50 650
11	Fe^{+++} Cit	26		1.8×10^{-6}	(−)6 480§
3	Fe^{+++} Cit	26		1×10^{-6}	1
3	$FeCl_3$	26		3×10^{-6}	48
6	Co Cit	27	9×10^{-6}‡	$1.7 \times 10^{-6} - 8.5 \times 10^{-6}$	311-480§
3	$CoSO_4$	27		3.5×10^{-7}	266
3	Ni Cit	28	6.1×10^{-7}‡	$1.7 \times 10^{-6} - 8.5 \times 10^{-6}$	311-480§
7	Cu Cit	29	6.3×10^{-7}‖	$1 \times 10^{-7} - 3 \times 10^{-7}$	(−)6-364§
19	$CuSO_4$	29		$1 \times 10^{-7} - 6 \times 10^{-7}$	50-318§
3	Zn Cit	30	1.6×10^{-6}‡	$1.5 \times 10^{-6} - 7.6 \times 10^{-6}$	311 480§
3	$ZnSO_4$	30		3.5×10^{-7}	266
3	KIO_3	53	7.1×10^{-6}†	4.6×10^{-6}	354
7	Re^0	75		5.5×10^{-6}	6-50
7	ReO_2	75		4.6×10^{-6}	(−)24-270§
3	Re_2O_7	75		9.7×10^{-6}	1
3	$NaReO_4$	75		2.7×10^{-6}	354

* These are estimates of the mouse's total body content prior to challenge. All estimates pertain to the individual metal involved in the test and not to the salt listed.
† Estimated from Hawk, Oser and Summerson (14).
‡ Estimated from Tipton and co-workers (18).
§ Include daily injections for one week.
‖ Estimated from Underwood (9).

2. Hughes and Cotzias (107) investigated the effect of glucocorticosteroids as compared with a mineralocorticosteroid on the transport of manganese. A total of 100 male Swiss albino mice (BNL strain; 7 weeks old; 18-20 g) were used in the experiments. In all experiments except one the isotope, carrier-free $^{54}MnCl_2$ in 20% HCl (diluted 20-50 fold with saline), was intraperitoneally injected (0.1 cc aliquots; 0.5-1.0 μc). In one experiment before intraperitoneal injection, the $^{54}MnCl_2$ was incubated with homologous mouse plasma so that it became plasma-bound. The hormones were subcutaneously injected in two ways: (a) 1 mg/day (50 mg/kg) as a daily dose of prednisolone and desoxycorticosterone acetate; and (b) the same dosage as aggregated doses every five days of desoxycorticosterone trimethylacetate and cortisol acetate.

It was found that glucocorticosteroids affected the tissue distribution of radioactive manganese in the mouse but mineralocorticosteroids had no effect. Livers retained less and carcasses more of the manganese tracer. The distribution of radiomanganese among the organs and the liver mitochondria was the same for the administration of either the plasma-bound or ionic form.

Because there is an induction period for the steroid effect, the authors considered that the steroid action on manganese metabolism was indirect as compared to that seen with metal loads (see this section, A 1).

3. Hughes et al. (108) investigated the possibility of endocrine regulation of manganese metabolism. A series of experiments was carried out using a total of 150 adult male mice, to study the effects

of administering ACTH or cortisol and also the effect of adrenalectomy on the excretion and tissue distribution of manganese (^{54}Mn and ^{55}Mn).

a. Two groups of six animals each were first intraperitoneally injected with carrier-free $^{54}MnCl_2$ (0.5-1.0 μc/mouse) and then injected daily with ACTH in gelatin (1 USPU/day) or gelatin alone for 30 days. Adrenal cortical stimulation with ACTH produced a shift of radiomanganese from the liver to the carcass, similar to that observed following glucocorticosteroid administration (see this section, A 2). This effect of ACTH was considered to suggest the existence of an adrenal regulatory mechanism in the metabolism.

b. Subcutaneous administration of cortisol acetate (approximately 50 mg/kg) into treated animals caused a shift in the tissue concentrations of ^{55}Mn from the liver and diaphragm to the carcass. The tissue concentrations of ^{55}Mn were changed by cortisol in the same direction as those of radiomanganese (^{54}Mn). The authors consider this to be proof that the glucocorticosteroid affected the metabolism of the essential metal manganese itself, and not merely that of its artificial tracer. The authors noted in this regard that since glucocorticoid hormones are administered to man in large amounts over long periods of time, they should be studied to see whether they induce syndromes in man related to experimental manganese deficiency.

c. When adrenalectomized animals were fed a wide range of manganese as $MnSO_4$ for two months, it was found that the manganese concentrations in the liver and diaphragm were altered only in those animals receiving high intakes of manganese. In this case the tissue

concentrations of manganese were higher. Thus both adrenalectomy and cortisol affected the tissue concentrations of manganese but the changes were in opposite directions.

d. Another finding was that administration of massive quantities of $MnCl_2$ resulted in a smaller increase in manganese tissue concentration when the adrenals were intact than when the animals were adrenalectomized.

From these observations the authors concluded that the metabolism of this essential trace mineral is apparently under regulatory control. The precise role of the adrenals in this control has not been established, however.

B. Rats

1. Papavasiliou et al. (194) examined the effect of obstruction of the rectum and the bile flow on the distribution and excretion of manganese. The experimental animals used were male albino Sprague-Dawley rats (200-250 g). Several different surgical methods were used for ligation of the common bile duct and rectum.

a. Groups of four rats with rectal obstruction were administered carrier-free $^{54}MnCl_2$ (1.0-1.5 μc in 0.1 ml 0.9% NaCl) intravenously, and $MnSO_4$ by intubation (doses: 3.2, 6.4 and 12.8 mg Mn^{2+}/animal). Rectal ligation was found to abolish the total body-loss of radio-manganese in both jaundiced and manganese-loaded rats. This confirmed the gastrointestinal excretion of manganese.

b. To study the contribution of bile to manganese excretion, biliary ligation was performed in animals administered the radio-manganese into either a peripheral vein or the portal vein. The effect

of bile duct obstruction was to decrease the excretion of radio-manganese. This was taken as proof that several gastrointestinal routes excrete manganese. When the ^{54}Mn was injected into the portal vein, excretion was very rapid. Finally, biliary ligation induced an initial rise followed by a decline of ^{54}Mn concentration in the liver, while the concentration of the stable metal ^{55}Mn was increased.

c. Further experiments showed that these observations were associated with the absorption and rapid excretion of stable manganese. By blocking the bile duct, the usual increased excretion following moderate loads of manganese was abolished. By overloading with manganese however, acceleration of excretion was induced.

To summarize these observations concerning the regulation of manganese excretion: (1) Excretion of manganese was abolished by rectal obstruction. (2) Biliary obstruction mainly impaired the sensitivity with which excretion was normally regulated. (3) Preventing the liver from receiving the initial bulk of tracer did not prevent its excretion, although the liver was accumulating instead of losing radioactivity.

The authors concluded that under ordinary conditions, absorbed manganese reaches the liver, becoming localized in the mitochondria, and while some of the manganese becomes distributed in the tissues, the largest fraction is discharged into the bile. Bile formation is therefore the main regulatory route under ordinary conditions, but when there is overloading or when the biliary route is blocked, excretion by auxiliary gastrointestinal routes takes place (see this section, B 2).

2. Bertinchamps et al. (015), continuing the line of research of several preceding papers, investigated the identification of various routes which excrete manganese and their possible interdependence in response to metabolic loads. The same procedures were followed with respect to rats, diets, housing, isotopes and concentration of dietary manganese described in the preceding paper (this section B, 1). The tributaries emptying into the cephalad segments of the intestine and the segments themselves were focused on as the significant excretory routes in this research.

Experiments were carried out to: (1) identify the intestinal excretory routes, (2) compare biliary and intestinal excretion, (3) measure the time sequences in the excretion of ^{54}Mn into the bile, and (4) determine the concentration of ^{55}Mn in the bile. (For experimental details see the original paper.)

Some of the observations were:

a. Much higher amounts of tracer were excreted by the duodenum and jejunum than by the terminal ileum.

b. The excretion via the cephalad segments could be accelerated sharply by manganese sulfate loading whereas excretion via the ileum could not. This suggested to the authors that the cephalad segments might be homeostatic end organs auxiliary to the liver.

c. There was a difference between the way intestinal and biliary excretion occurred. The radiomanganese was excreted into the bile in two distinct successive waves. The first wave was apparently connected to the passage of tracer from the plasma into the bile in the direction of a steep concentration gradient

which increased during the second wave. The second wave reflected an acceleration of the enterohepatic circulation of manganese. It was present when the animals consumed a high manganese diet (0.002%).

The authors concluded that when the enterohepatic circulation of manganese is saturated by overloading, excretion of manganese takes place via the duodenum and jejunum.

C. Cows

Fain et al. (068) investigated the effect of varying amounts of Mn on the glucose, magnesium, iron and potassium levels in cattle blood. Manganese sulfate was added to the silage of five pure bred Hereford cows. The manganese content of the dietary components was first determined, then $MnSO_4$ was added in increasing amounts to raise the total manganese content to 75 ppm, 100 ppm, 150 ppm and 200 ppm. This was done over an experimental period of one and a half years. Blood samples were taken at intervals from the external jugular vein.

There were no signs of the onset of symptoms of tetany and no evidence of hyperexcitability. The most noteworthy observations were the simultaneous decrease of magnesium in all the animals at 100 ppm and the subsequent rise at 150 ppm. The authors questioned whether continued intake of Mn at the level of 100 ppm would produce still lower magnesium values and eventually result in tetany. There was no observed significant effect on the concentration or metabolism of glucose, iron, calcium or potassium at the given amounts of supplemental dietary manganese.

D. Humans

Borg and Cotzias (025) studied blood clearance and radiation measured at the body surface following ^{56}Mn injection in man. Fourteen patients were intravenously injected with ^{56}Mn. This was followed by analysis of their blood clearance and liver uptake.

It was found that the injected radiomanganese disappeared rapidly from the blood stream (see Figure 7). The authors resolved this clearance into three phases. The first and fastest of these is identical with the clearance rate of other small ions, suggestive of the normal transcapillary movement; the second phase is identified with the entrance of manganese into the mitochondria of the tissues; and the third phase which is the slowest is considered to be indicative of the rate of nuclear accumulation of manganese. The above interpretations are supported by previous research from the same laboratory with radiomanganese, which demonstrated early and preferential accumulation in the mitochondria-rich organs of the body, localization of manganese in the mitochondria of the cell, and high mitochondrial but low nuclear turnover rates.

The kinetic patterns for blood clearance and liver uptake of manganese were found to be almost the same, thus indicating that the two manganese pools, blood manganese and liver mitochondrial manganese, rapidly enter equilibrium. The authors concluded therefore that a high proportion of body manganese must be in a dynamic, highly mobile state.

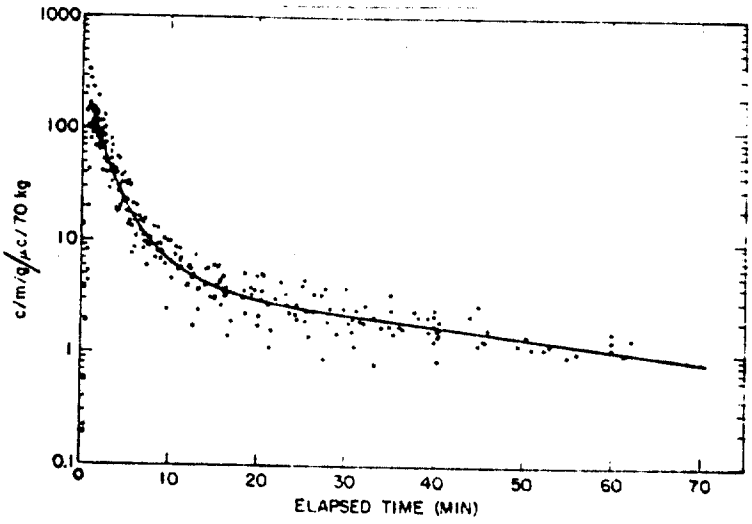


Fig. 7. Clearance of Mn^{56} from the blood after ^{56}Mn I.V. (humans) (025)

IV. Effect on Enzymes and Other Parameters

Introduction

Cotzias (045) summarizes the enzymes and enzyme systems in which manganese plays a role.

1. Manganese specifically activates prolidase.

2. Manganous ions are thought by several researchers to be required for oxidative phosphorylation.

3. Manganese plays a major role in the metabolism of fatty acids *in vivo*.

The author concluded that research results point to the importance of manganese in mitochondrial function. He noted in this regard that it has been found that the structure of isolated mitochondria is preserved by the addition of trace amounts of manganese.

Mikhaylov (170) pointed out in his review that his own research has shown manganese to be an acetylcholinesterase inhibitor. He also noted that manganese compounds are powerful oxidizing agents which oxidize catecholamines almost instantly. In addition, manganese also activates enzymes which split catecholamines, in particular MAO. Under certain conditions, however, it suppresses their activity, i.e. when the protein structure of the granules in which the catecholamines are deposited in the cell is injured.

The author also reported research showing that prolonged (nine months) intravenous injection of $MnCl_2$ produced a sharp decrease in the protein sulfhydryl groups in rabbit's blood. He also reported research results showing that following administration of this com-

pound, the metabolism of catecholamine, serotinin, acetylcholine and histamine was reported to be changed as well as the content of RNA and purine bases in both the nerve cells and the neuroglia.

A. In Vitro

1. Iwabuchi (113) found that of nine tested metal salts, only $MnSO_4$ activated arginase. When 1 cc M/100, M/500 or M/5000 $MnSO_4$ was added to arginase prepared from a dialyzed autolyzate of pig liver for one hour at 37° and pH 9.2, the activity increased 30, 26 and 21% respectively.

2. Shimatani (227) reported on the influence of several chemicals including $MnCl_2$ which are known to be activators or inhibitors of phosphatase, on both the dephosphorylating (hydrolyzing) and phosphorylating action of this mucosa enzyme. The source of the enzyme phosphatase was a glycerol extract of the acetone powder prepared from the mucosa of a rabbit's small intestine. As can be seen in Table 33, $MnCl_2$ inhibited the dephosphorylating action of the intestinal mucosa in concentrations of 50M to 200M. At a concentration of 50M, the phosphorylation action was also inhibited.

3. Scrutton et al. (223) found that pyruvate carboxylase is a manganese metalloprotein. The metal content of the enzyme was determined by various methods to be 2.5 to 4.3 moles of manganese per mole of enzyme. The magnetic properties of the protein-bound manganese were found to be consistent with the divalent manganese ion.

4. Mildvan et al. (171) investigated the role of the tightly bound manganese in the metalloprotein, pyruvate carboxylase (see this section, A 3). From their experiments (see original paper for

Table 33. Influence of $MnCl_2$ on the Dephosphorylating and Phosphorylating Actions of the Intestinal Mucosa (glycerol extract (1:30)) (227)

Hours	Final M conc. of $MnCl_2$	0 (control)	M/200	M/100	M/50
	Hydrolysis:	Increase of inorganic P. (mg in 1.0 ml digest)			
2		0.027	0.021	0.012	0.012
5		0.053	0.039	0.029	0.023
24		0.117	0.090	0.062	0.051
	Synthesis:	Decrease of inorganic P. (mg in 1.0 ml digest)			
24		0.09	0.07	0.06	0
72		0.20	0.20	0.21	0.11
120		0.29	0.25	0.30	0.13

details), the authors concluded that the manganese functions in the transcarboxylation part of the pyruvate carboxylase.

B. Rats

1. Amdur et al. (002) showed that manganese supplements reduced liver and bone fat in Mn-deficient rats. Six groups of rats (31 per group) were fed the following six diets respectively: (a) low choline (0.1 mg/g), low manganese (0.3 γ/g); (b) medium choline (0.4 mg/g), low manganese; (c) high choline (0.8 mg/g), low manganese; and three high manganese diets, one for each choline level, which were obtained by supplementing the corresponding low manganese diet with 0.25% $MnCl_2$.

The results are summarized in Fig. 8. It was found that manganese prevents the deposition of excess fat in the liver. At a given level of choline there was more fat found in the livers of manganese-deficient rats than in those with adequate dietary manganese. The lipotropic action of manganese was much greater with low choline diets, this was interpreted as indicating an interaction between manganese and choline. As further evidence for the lipotropic action of manganese, it was found that when there was supplementary manganese in the diet there was a highly significant reduction in the percentage of fat occurring in fresh bone.

2. Gubler et al. (090) determined whether or not the administration of large amounts of manganese influenced copper metabolism in animals. The experiments were carried out with 83 male, weanling Sprague-Dawley rats divided into four groups. The general experimental design, schedule of intraperitoneal injections and growth curves are shown in Fig. 9.

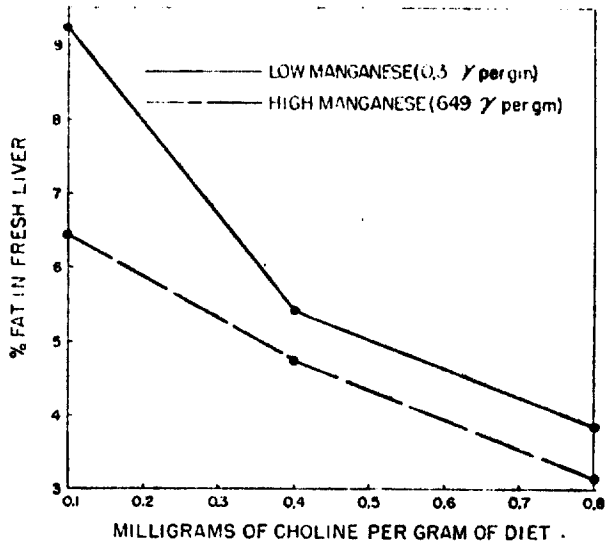


Fig. 8. The action of manganese and choline on liver fat (002)

It was found that the administration of manganese was associated with: (a) A significant increase ($P<0.01$) in copper concentration in the plasma and brain. (b) A decrease in urinary copper excretion. (c) No significant alteration in copper concentration in the liver. (d) A decrease in kidney copper concentration. (e) A significant decrease ($P<0.01$) in the total amount of iron in the body. (f) A significant decrease ($P<0.01$) in the amount of iron per 100g BW. (g) A mild, microcytic and slightly hypochromic anemia accompanied by a slight increase in reticulocytes developed. (h) The total amount of copper in the body was not increased.

When large amounts of manganese and copper were administered simultaneously it was found that: (a) There was a marked increase in total body copper twice that of rats given the same amount of supplemental copper alone. (b) There was an increase in copper concentration

in the plasma, liver, kidneys and brain. (c) The same anemia described above developed. (d) The concentration of copper was increased five-fold.

The authors suggested that manganese may form a complex with copper making it unavailable. Also that it may somehow block the action of copper-containing enzymes.

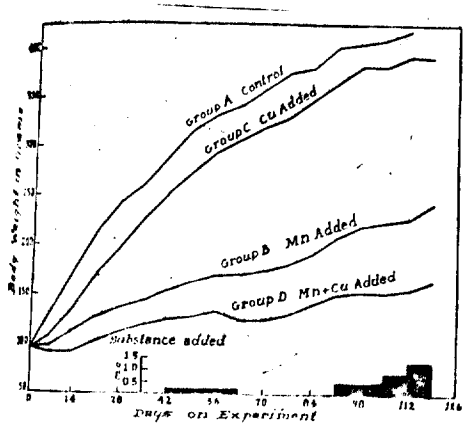


Fig. 9. Influence of manganese, copper, and copper in addition to manganese on growth of rats. All animals received the basal diet and the following supplements: Group A, none; group B, 4% manganese chloride; group C, 0.1% copper sulfate; group D, 4% manganese chloride and 0.1% copper sulfate. In addition, the animals in group B were given manganese chloride ($MnCl_2 \cdot 4\ H_2O$) intraperitoneally in amounts indicated; those in group C were given "Cupralene" in this way and those in group D, both manganese chloride and "Cupralene." These compounds were injected 3 times weekly. Half the animals in each group were sacrificed at 86 days, the remainder at 120 days. As a result those sacrificed at 86 days received a total of 4.5 mg $MnCl_2$ intraperitoneally while the remainder received a total of 25.5 mg. The total amounts of "Cupralene" received by the animals sacrificed at these different times were also 4.5 and 25.5 mg, respectively. (090)

3. Conrad and Baxter (041) determined the effect of $MnCl_2$ on the Q-T interval and the uptake and distribution of ^{45}Ca by the myocardium. This study was prompted by a previous finding from the same laboratory that manganese excess in rats results in a prompt fall in serum calcium and the fact that a decrease in serum calcium concentration is associated with a prolonged Q-T interval.

Holtzman albino rats (224 ± 21 g, number not given) were first subcutaneously injected with 0.5 ml ^{45}Ca solution (as $^{45}CaCl_2$). Then one-half of the animals were subcutaneoulsy injected with 60 mg $MnCl_2$ in 0.25 ml demineralized water. Then the experimental animals were again divided in half, and one group was sacrificed 2 hours and the other 24 hours following manganese administration.

It was found that in the manganese-treated rats:

a. There was a fall in serum concentration from 5.34 ± 0.52 to 4.32 ± 0.4 mEq/liter ($P<0.01$) within 24 hr.

b. The Q-T interval was prolonged at 2 and 24 hours ($P<0.001$).

c. The total uptake of ^{45}Ca was doubled at 2 hours due to increases in the microsomal and 17 hour fractions.

d. A significant decrease in serum calcium concentration was observed 24 hours following Mn administration.

The authors concluded that the administered manganese had disrupted physiologic mechanisms governing both the entry of calcium into the myocardial cell and its distribution.

4. Voynar and Galakhova (267) studied the effect of manganese (as $MnCl_2$) on the quantity of fat and phospholipids in the livers of carbon tetrachloride (CCl_4) poisoned animals. Forty-five white rats

were divided into four groups:

a. The first group were normal, control animals.

b. The second group were given only CCl_4 injections (0.4 ml/100 g BW on four or five successive days), then sacrificed on the fifth or sixth day from the start of the experiment.

c. The third group were subcutaneously injected with $MnCl_2$ (0.7 mg/kg BW for 15 days) and killed with a CCl_4 injection at the end of the dose period (15th or 16th day).

d. The fourth group, rats which received CCl_4 (0.4 ml/100 g BW) from the start of the experiment, were divided into two subgroups; one subgroup received $MnCl_2$ at the above dose for 15 days following CCl_4 poisoning and the other subgroup did not receive $MnCl_2$. All these animals were sacrificed on the 20th or 21th day from the start of the experiment. The experimental results are summarized in Table 34.

The results showed that:

a. The animals given $MnCl_2$ before CCl_4 poisoning (group 3) showed some increase in the liver content of fat and phospholipids. The data indicates that whereas prior administration of $MnCl_2$ does not completely prevent fatty infiltration in the liver, it does help reduce the total quantity of deposited fat.

b. In those animals given $MnCl_2$ after CCl_4 poisoning (group 4), the amount of fat and phospholipids decreased about two-fold and six-fold respectively compared to group 2 (no manganese).

c. Compared to controls, the group 3 animals ($MnCl_2$ before CCl_4) showed a slightly lower than normal liver fat content and a three-fold reduction in phospholipids.

Table 34. Content of Total Lipids and Lipid Phosphorus in the Livers of the Control Rats and Rats Poisoned with CCl_4 and the Quantitative Changes in These Substances After Subcutaneous Injection of Manganous Chloride (267)

Indices determined	Groups of animals				
	Control (I)	Poisoned with CCl_4 (II)	Previous given $MnCl_2$	Poisoned with CCl_4 (IV)	
				Given $MnCl_2$	Not given $MnCl_2$
Total lipids in wet weight, %	4,8±0.17	13.0±0.99 < 0.001	11.6 ± 0.98 < 0.01	4.1 ± 0.6 < 0.001	7.0 ± 1.16 < 0.001
Lipid phosphorus, mg%	75.0±10.4	212±21.2 < 0.001	203 ± 14.1 < 0.5	24.0 ± 3.0 < 0.001	100 ± 7.9 < 0.001
Moisture content	70.4±0.76	67.4±1.2 < 0.001	57.4 ± 1.2	71.8 ± 1.0 < 0.001	71.0 ± 1.1 < 0.001

The authors concluded that manganese compounds increase the liver's ability to eliminate fat.

5. Mosendz and Silakova (178) studied the effect of manganese chloride on ammonia metabolism in the brain and other tissues. Rats (200-220 g, number, strain and sex not given) were intratracheally injected with 10% $MnCl_2$ at the rate of 15 mg Mn/rat. Controls were similarly administered physiological saline. All the animals were sacrificed 1, 6, or 30 days after poisoning. The tissues were studied for ammonia and amide protein nitrogen content. (See figures in original paper for details of the results.)

It was found that under the experimental conditions, there was a sharp increase in the amount of ammonia in all the tissues studied. There was not an identical increase in tissue ammonia content, however. The increase was greatest in the liver. At the same time as the ammonia accumulates in the tissues, it was observed that the processes which neutralize its toxicity are also intensified, i.e. the amount of both glutamine and amide nitrogen in proteins also increased. The synthesis of glutamine (amidation of free glutamic acid) was found to be particularly increased in all the tissues.

C. Guinea Pigs

1. Frommel et al. (078) studied the effect of manganese chloride on the serum cholinesterase of guinea pigs in vivo. The salt was subcutaneously injected into two animals. The serum enzymatic activity was measured by the titrimetric method of Hall and Lucas both before and after injection. The experimental data and results are shown in Table 35. Manganese chloride, which was not found to be active

Table 35. Effect on Manganese Chloride on the Serum Cholinesterase of Guinea Pigs In Vivo (078)

Compound	Guinea pig	Weight	*Dose received in g/kg	CHE before	CHE after	Result in %	Average
Manganese chloride	m	505	4 x 0,01	2,01	1,10	-45%	
	m	400	4 x 0,01	1,55	1,10	-29%	-37%

*The injected dose was calculated according to Aberhalden (Handbook of Biological Work Methods 1, 7) to obtain the chronic toxicity for the ion studied.

in vitro on horse serum in a previous experiment by these researchers, was found to inhibit cholinesterase in guinea pig serum *in vivo*.

2. Everson and Schrader (067) produced direct evidence that manganese is involved in glucose utilization. They investigated the physiological response of manganese-deficient, deficient-supplemented, and control guinea pigs to orally and intravenously administered glucose loads. The animals used in the study were the offspring of female guinea pigs fed diets containing either less than 3 ppm Mn or 125 ppm Mn as $MnSO_4$.

The experimental results are summarized in Figures 10, 11, and 12. The manganese deficient animals showed decreased glucose utilization reflected in a diabetic-like glucose curve in response to glucose loading. Controls and manganese-deficient animals administered dietary manganese equivalent to the 2-month control diet showed normal responses to glucose administration.

The authors concluded that their studies showed that manganese supplementation completely reversed the previously reported reduced glucose utilization in manganese-deficient guinea pigs.

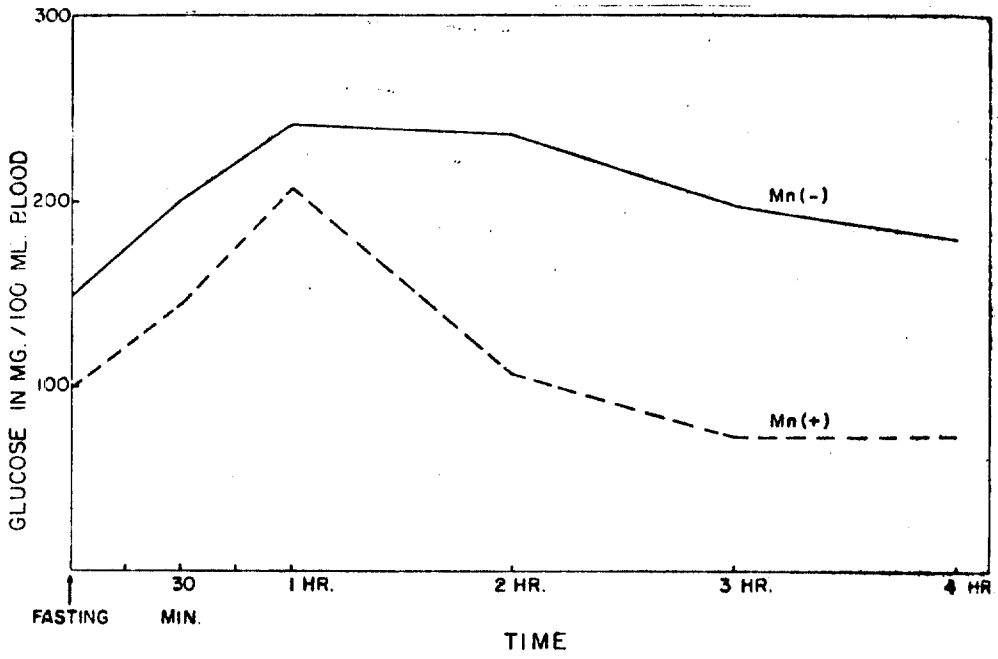


Fig. 10. Glucose tolerance curves of guinea pigs. (Oral administration of glucose, sodium pentobarbital anesthesia, blood sampling by nail-bed clipping.) (067)

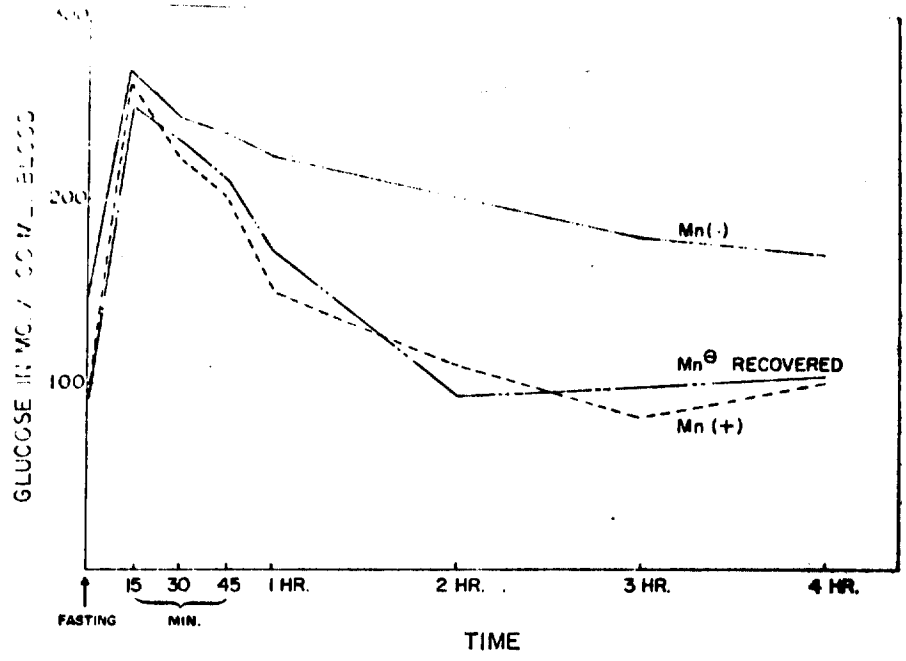


Fig. 11. Glucose tolerance curves of cannulated guinea pigs. (067)

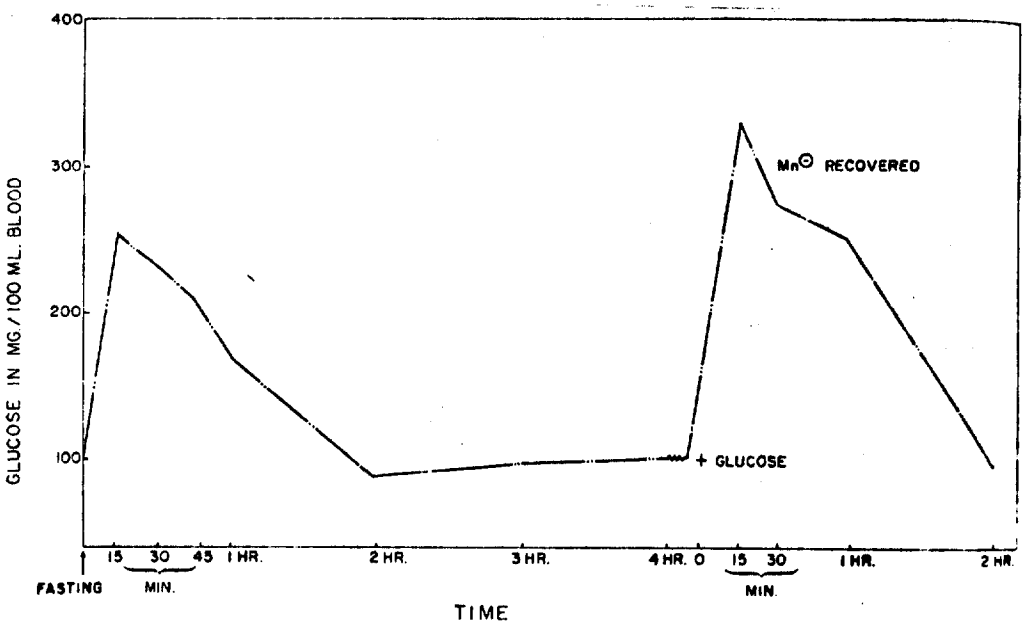


Fig. 12. Glucose tolerance curves of supplemented guinea pigs following repeated administration of glucose via cannula placed in the carotid artery. (067)

3. Maksimov and Laskavaya (153) studied the effect of various doses of $MnCl_2$ on the glucocorticoid function of the adrenal cortex. Four experiments were carried out using a total of 140 guinea pigs (260 to 320 g). In the first three experiments (86 animals), the plasma concentration of 17-hydroxycorticosteroids was studied after intramuscular administration of $MnCl_2$ in three doses 3, 10, and 100 µg $MnCl_2$/animal. Controls were similarly administered physiological saline. The results are shown in Tables 36, 37, and 38.

In the fourth experiment (54 animals), the ascorbic acid content of the adrenal tissue after administration of 100 µg $MnCl_2$ was studied (see Table 39).

It was found that:

Table 36. Change in Concentration of 17-Hydroxycorticosteroids ($\bar{x} \pm S\bar{x}$) in Plasma After Administration of 3 μg of Manganous Chloride (153)

Animals in series 1	No. of animals	Duration of administration of manganous chloride (in days)	Amount of 17-hydroxycorticosteroids (in μg/100 ml of plasma)						
			Original level	After 10 injections	P	After 15 injections	P	7 days after end of administration	P
Group 1	9	10	59.70±2.2	75.72±3.4	< 0.01			65.66±2.35	< 0.1 > 0.05
Control	7		50.48±1.86	54.71±3.78	> 0.03			46.44±3.91	> 0.05 > 0.03
Group 2	8	15	29.14±6.27			53.52±3.31	< 0.01	74.34±4.76	< 0.001
Control	9		39.03±2.72			40.73±4.35	> 0.7	39.89±2.01	> 0.8

Table 37. Change in Concentration of 17-Hydroxycorticosteroids ($\bar{x}\pm S\bar{x}$) in Plasma after the Administration of 10 μg of Manganous Chloride (153)

Animals in series 2	No. of animals	Duration of admin. of manganous chloride (in days)	Amount of 17-hydroxycorticosteroids (in μg/100 ml of plasma)											
			Original level	After 5 inject.	P	After 10 inject.	P	After 15 inject.	P	After 20 inject.	P	7 days after end of admin.	P
Group 1	8	10	48.90±3.91			58.10±7.23	>0.2		<0.1			51.76±4.59	>0.6
Group 2	8	15	38.51±2.36			40.28±4.50	>0.7	50.14±5.90	>0.05			51.24±4.68	<0.05
Group 3	10	20	51.28±2.80	58.36±5.30	>0.7			65.33±6.07	0.05	50.79±6.09	>0.9		
Control	9		39.03±2.72	42.60±1.60	>0.2	46.91±3.25	>0.05	40.73±4.35	>0.7	41.59±2.38	>0.4	39.89±2.01	>0.8

Table 38. Change in Concentration of 17-Hydroxycorticosteroids ($\bar{x}\pm S\bar{x}$) in Plasma After the Administration of 100 μg of Manganous Chloride (153)

Animals in series 3	No. of animals	Duration of admin. of manganous chloride (in days)	Amount of 17-hydroxycorticosteroids (in μg/100 ml of plasma)								
			Original level	After 5 Inject.	P	After 10 inject.	P	After 20 inject.	P	4 days after end of admin.	P
Group 1	10	20	42.63 ± 3.25			45.19 ± 5.27	>0.6	28.28 ± 4.75	<0.05	41.85 ± 3.37	>0.8
Group 2	10	10	52.64 ± 3.39			53.22 ± 3.34	>0.9				
Group 3	7	10	55.52 ± 8.67	94.41 ± 9.32	<0.02	56.02 ± 6.85	>0.9				
Control	9		39.03 ± 2.72	42.60 ± 1.60	>0.2	46.91 ± 3.25	>0.05	41.59 ± 2.38	>0.4		

Table 39. Effect of Administration of 100 µg of Manganous Chloride on the Ascorbic Acid Content of Guinea Pig Adrenals (153)

Animals in series 4	No. of animals	Duration of admin. of manganous chloride (in days)	Ascorbic acid content (in µg per weight of organ)	P
Group 1	9	5	21.02 ± 1.01	< 0.02
Control	9		31.30 ± 3.84	
Group 2	9	10	29.17 ± 2.06	< 0.1
Control	9		36.68 ± 2.93	> 0.05
Group 3	11	20	34.69 ± 2.07	
Control	7		28.32 ± 1.88	< 0.05

a. In the animals receiving 3 μg $MnCl_2$ for 10 or 15 days, the amount of 17-hydroxycorticosteroids increased in the peripheral blood plasma.

b. In the animals receiving 10 μg $MnCl_2$ for 10, 15 and 20 days, the amount of steroids in the plasma tended to increase after 15 days.

c. In the animals receiving 100 μg $MnCl_2$ for five days, the steroid concentration was increased. When given for a longer period (20 days) the steroid concentration was reduced and there was no effect noticed after ten days of administration at this dose level.

d. Daily administration of 100 μg $MnCl_2$ for one to five days lowered the ascorbic acid content of the adrenals below control values ($P<0.02$).

The authors concluded that: (a) Their data showed that $MnCl_2$ administration may alter the glucocorticoid function of the adrenal cortex. (b) The dosage at which $MnCl_2$ affects the 17-hydroxycorticosteroid plasma level also alters the amount of ascorbic acid in the adrenals. Therefore changes in the vitamin C level of the adrenals which are induced by $MnCl_2$ reflect to some degree changes in corticosteroid secretion.

D. Monkeys

Neff (179) studied the biogenic amine concentration in monkey brain after chronic administration of MnO_2. A suspension of MnO_2 in olive oil (200 mg MnO_2/ml; a total of 1 ml used) was subcutaneously injected at several sites into 15 squirrel monkeys. Five controls received 1 ml olive oil.

It was found that the concentration of caudate nucleus dopamine

was significantly reduced. Caudate nucleus concentrations of serotinin were also reduced. The author concluded that since manifestations of extrapyramidal motor dysfunction resulting from MnO_2 administration were associated with a deficiency of dopamine and serotonin in the caudate, this suggests that this deficiency may play a significant role in producing this dysfunction.

E. Humans

1. Rodier (216) carried out an exhaustive biological study of 151 patients with manganese poisoning. (See original paper for details of the tests made.)

The following observations were made:

a. Renal: There did not seem to be any serious disturbance, but 40% of the patients retained dye in the phenol sulfonaphthalene test.

b. Hepatic: The Maillard coefficient was altered in 61% of the patients.

c. Endocrine: This examination showed the largest alterations. The elimination of 17-ketosteroids was decreased in 80% of the subjects. The basal metabolism was increased in 53% of the patients.

d. Hematology: In 41%, of the patients there was an unquestionable inversion of the leukocytic formula in the direction of a lymphocytosis. There was a slight modification in 29.5%. In 44% the blood corpuscle count indicated a slight polycythemia.

The author concluded that these tests should be carried out on all manganics: (1) the blood cell count, (2) the establishment of the leukocytic formula, (3) the measurement of basal metabolism, (4)

determination of the amount of urinary ketosteroids eliminated.

2. Rubenstein et al. (218) reported the case of a hypoglycemic effect on a diabetic following infusions of lucerne (alfalfa, Medicago sativa), a plant with a high manganese content. The patient was then administered manganese chloride orally (3-5 mg) and one time an intravenous injection of 20 mcg. There was a consistent fall to severely hypoglycemic levels. Oral administration of either lucerne or manganese modified the glucose tolerance curve.

The authors suggested that the time-interval between administration of the manganese and its effect on the blood glucose-level is consistent with the possibility of enzyme activation.

V. Drug Interaction

A. Mice

1. Ceasar and Schnieden (034) examined the effect of manganese sulfate on the toxicity and tremor caused by temorine in mice. To study the effect on tremorine toxicity, $MnSO_4$ was first intraperitoneally injected, then 25 minutes later tremorine was injected by the same route into 16 male albino mice (TT strain, 17-30 g). Mice were injected either with 0.9% saline and tremorine (controls), $MnSO_4$ alone or tremorine and $MnSO_4$. The results are summarized in Table 40 which shows $MnSO_4$ had a marked potentiating effect on the toxicity of tremorine.

To study the effect on tremorine tremor, $MnSO_4$ (100 mg/kg) was intraperitoneally injected 15 minutes prior to the tremorine injection. The results are summarized in Table 41. The salt itself did not cause tremor nor did it seem to interfere with the characteristic autonomic effects of tremorine.

The authors suggested that chelation of the manganese with aromatic amines is a possible explanation for the potentiating action on the toxicity of the tremorine.

2. Mikhaylov (170) reported in his review on the pathogenesis and therapy of manganese poisoning that his own research had shown that chlorpromazine, neostigmine and reserpine potentiate the toxic effect of $MnCl_2$ in white mice. He concluded from his results and the work of others that the increased concentration of manganese in brain tissue may play a part in the mechanism of this phenomena. Experiments

Table 40. The Effect of Copper Sulphate and Manganese Sulphate on the 1-hr LD_{50} of Tremorine (034)

Treatment	Dose	No. of animals used	No.dead after tremorine	% Death
0.9% saline; tremorine 25 min later	0.25 ml 315 mg/kg	16	8	50
Manganese sulphate; tremorine 25 min later	300 mg/kg 315 mg/kg	16	15	93.75

Table 41. The Effect of a Previous Injection of Copper Sulphate or Manganese Sulphate on Tremorine Tremor (034)

Treatment No.	Mean tremor score at times after tremorine or saline			
	10 min	15 min	20 min	40 min
Tremorine (30 mg/kg) (4)	19.52	14.89	17.67	6.87
Manganese sulphate (100 mg/kg) 15 min later tremorine (30 mg/kg)(5)	26.10	21.30	21.53	15.08
0.25 ml 0.9% saline (6)	6.63	7.80	9.08	7.75

on monkeys have shown that the amount of manganese in the basal ganglia increases sharply after peroral or intramuscular administration of chlorpromazine. This has been found to correlate with the intensity of clinical neurologic disorders.

3. The experiment of Hughes et al. (108) in which a glucocorticosteroid, cortisol acetate, was administered to mice is described in detail in Biochemical Section III A 3. It was found that in radiomanganese injected animals, subcutaneous injection of cortisol acetate (approximately 50 mg/kg) caused a shift in the tissue concentrations of stable manganese in the same direction as that for the radioactive isotope. The authors considered this to be proof that the glucocorticosteroid affected the metabolism of the essential metal manganese itself.

In this regard the authors noted that since glucocorticoid hormones are administered to man in large amounts over long periods of time, they should be studied to see whether they induce syndromes in man related to experimental manganese deficiency.

B. Guinea Pigs

Pico (202) investigated the influence of manganese (as $MnCl_2$) on the strength of antitoxin serums. The following were studied:

1. Anti-diphtheria serum: Ten days after immunization of a horse, 10 cc of M/2 $MnCl_2$ solution was intravenously injected for three consecutive days. Manganese was found to raise the antitoxin level by 17%.

2. No change was produced by $MnCl_2$ in the strength of the tetanus antitoxin, the precipitant serums, the hemolytic serums

or the antiophidica serums.

3. In the case of anti-meningococci serum, $MnCl_2$ seemed to increase the fixative power of alexin but there were no clear results with agglutination.

4. When 35 sensitized male guinea pigs were injected with 0.5 cc $MnCl_2$ (4 parts per 1000) immediately preceding (1 to 4 min) the injection of a strong dose of shock antigen (1 cc horse serum), one-third were almost completely protected.

C. Chicks

Comens (039) found that when one group of ten-day old cockerels (number not given) was fed 10 mg per day of hydralazine, all developed perosis within six weeks. However, when another group was simultaneously fed 10 mg per day hydralazine plus 6 mg per day manganese citrate their development was normal. The author concluded from this and other experiments with dogs and humans (see below) that hydralazine may produce "hydralazine disease" by binding the manganous ion, thus possibly blocking dependent enzyme systems.

D. Dogs

Comens (039) reported the induction of "hydralazine disease" in dogs with lupus erythmatosus cells in their blood which were fed hydralazine alone. When manganous citrate was administered parenterally with the hydralazine to two dogs, no "glomerular wire loops" appeared. The author concluded from this and other experiments with chicks and humans that the drug hydralazine may produce "hydralazine disease" by binding manganous ion, thus possibly blocking dependent enzyme systems.

E. Humans

1. Comens (039) found that three patients with "hydralazine disease" and two with disseminated lupus apparently improved symptomatically when manganous ion was administered. This improvement occurred even with the continuation of hydralazine administration. The author concluded from these results along with evidence from experiments with chicks and dogs that the drug hydralazine may bind manganous ion thus possibly blocking dependent enzyme systems.

2. Shugaylo and Gude (235) found that the combined administration of $MnCl_2$ with vitamins C and B_1 was beneficial in the treatment of patients with infectious hepatitis. They divided 94 patients into two groups. One group (49 persons) was orally treated with manganese (0.1 to 0.05% $MnCl_2$ solution, 10 to 20 mg daily for two weeks) and vitamins C and B_1. The other group (45 persons) was given only the vitamins.

It was found that the $MnCl_2$ treated patients showed a more rapid lowering of the pyruvic acid level in their urine and consequently a more rapid normalization of carbohydrate metabolism. Their general condition was found to improve sooner with a return of appetite and a more rapid reduction in pain. The authors concluded that manganese in conjunction with vitamins C and B_1 alleviated the symptoms of infectious hepatitis more quickly than vitamin therapy alone.

VI. Consumer Exposure

Manganese is found in all foodstuffs of animal and vegetable nature. Table 1 presents the occurrence level of manganese in some common food categories based on the work of Peterson and Skinner (201). There has been substantial detail work done on determining manganese levels in specific fruits, vegetables, meats, and seafoods. Predominant among these are the studies published during the late 1920's and early 1930's from the Department of Agricultural Chemistry at the University of Wisconsin: Lindow and Peterson (147), Peterson and Skinner (201,242,243) and Hodges and Peterson (103). Others include Remington and Shiver (212), who studied the manganese, iron and copper content of some common vegetables; Davidson (051), who investigated manganese levels in cereals and cereal mill products; and Czerniejewski *et al.* (050) who dealt with wheat, flour, and bread.

Average daily intakes of manganese from the trace amounts reported in food have been estimated. These are reviewed by Underwood (262). Estimates range from 2.3 to 6.4-7.5 mg Mn/day for adults.

Since the manganese to which a consumer is exposed from natural food sources is most likely in a chelated form, the amount taken in from such sources should be considered separately from manganese salts which are added to food. The observation that the transport across cell walls is a function of the properties of the individual complex led Cotzias to state that "there is no necessary correlation between the rate of absorption of large amounts of inorganic manganese salts and that of trace amounts occurring in foods" (044). Therefore, studies done on the ingestion of manganese salts which have been added to the diet are of specific significance both from a biological standpoint and from the standpoint of the function of this monograph.

At present, several manganese salts appear on the GRAS list. Of those treated in this monograph, the Food Chemicals Codex (040) lists manganese chloride, manganese gluconate and manganese sulfate as nutrients and dietary supplements. The NAS/NRC Comprehensive GRAS Survey of 1972 (073) showed a total 1970 poundage reported to NAS and FEMA of 10 for manganese chloride and 3,432 for manganese sulfate. The same NAS/NRC report gives the average amount (weighted mean) added to baby food of manganese chloride as 313 ppm and of manganese sulfate as 23.7 ppm.

Table 42 shows usage levels for manganese sulfate from the NAS/NRC report. Table 43 lists possible daily intakes of manganese chloride and manganese sulfate.

Manganese in an inorganic form may also be encountered in drinking water. In 1962 the United States Public Health Service (004) set the maximum level of manganese in drinking water at 0.05 mg/liter. Although manganese usually occurs as dissolved carbonate at levels near 2 mg/liter in raw groundwater, a study has shown that about 80% of the municipal groundwater in Illinois contained less than the maximum set by the USPHS (185). Removal of manganese where it does occur at high levels is commonly done through precipitation of the oxide following aeration, or by ion-exchange.

Table 42 - Usage Levels Reported for NAS Appendix A Substances (group I) used in Regular Foods (R) (073)

Substance name (survey No.)	Food category No.	Name	# Firms Reporting	***Usual use MTD Mean, %	******Maximum use MTD Mean, %
Manganese sulfate NAS. 0124	01	Baked goods (R)	*	.02700	.02700
	05	Milk Prods(R)	*	.00067	.00095
	10	Meat Prods(R)	*	.00060	.00060
	11	Poultry (R)	*	.00060	.00060
	13	Fish Prods(R)	*	.00060	.00060
	22	Snack Foods (R)	*	.00000	.00000
	23	Bev Type I(R)	*	.00000	.00000
	28	Imit Dairy(R)	*	.00550	.00550

Table 43. Comprehensive GRAS Survey - NAS/NRC 1972 (073)

Substance Name (survey No.)	Food Category (No. Name)	# of firms	Age	Possible Daily Intake Mg. Average	High A	High B
Manganese Chloride NAS 0119	83 Formulas (B)	*	0-5 Mo.	105.074100	192.495000	**********
			6-11 Mo.	21.409200	102.006700	**********
			12-23 Mo.	6.886000	1.940600	**********
Manganese Chloride NAS 0119	All Categories ********** **********	*	0-5 Mo.	105.074100	192.495000	**********
			6-11 Mo.	21.409200	102.006700	**********
			12-23 Mo.	6.886000	1.940600	**********
Manganese Sulfate NAS 0124	01 Baked Goods (R)	*	0-5 Mo.	.918000	1.215000	.918000
			6-11 Mo.	6.858000	13.986000	6.858000
			12-23 Mo.	14.715000	24.246000	14.715000
			2-65+ Yr.	37.044000	55.026000	37.044000
Manganese Sulfate NAS 0124	05 Milk Prods(R)	6	0-5 Mo.	.036180	.026800	.051300
			6-11 Mo.	.418080	2.010670	.592800
			12-23 Mo.	.365150	1.168480	.517750
			2-65+ Yr.	.264650	.808020	.375250
Manganese Sulfate	10 Meat Prods(R)	*	0-5 Mo.	.006600	.017400	.006600
			6-11 Mo.	.124200	.334800	.124200
			12-23 Mo.	.181200	.311400	.181200
			2-65+ Yr.	.470400	.780600	.470400
Manganese Sulfate NAS 0124	11 Poultry(R)	*	0-5 Mo.	.003000	.013800	.003000
			6-11 Mo.	.023400	.079200	.023400
			12-23 Mo.	.039600	.110400	.039600
			2-65+ Yr.	.077400	.196800	.077400
Manganese Sulfate NAS 0124	13 Fish Prods(R)	*	0-5 Mo.	.000600	.001800	.000600
			6-11 Mo.	.007800	.029400	.007800
			12-23 Mo.	.032400	.081000	.032400
			2-65+ Yr.	.074400	.185400	.074400
Manganese Sulfate NAS 0124	22 Snack Foods(R)	*	0-5 Mo.	**********	.000000	**********
			6-11 Mo.	.000000	.000000	.000000
			12-23 Mo.	.000000	.000000	.000000
			2-65+ Yr.	.000000	.000000	.000000

Table 43. Comprehensive GRAS Survey -- NAS/NRC 1972 (Cont'd)

Substance Name (Survey No.)	Food Category (No. Name)	# of firms	Age	Possible Daily Intake, mg. Average	High A	High B
Manganese Sulfate NAS 0124	23 Bev Type 1(R)	*	0-5 Mo.	.000000	.000000	.000000
			6-11 Mo.	.000000	.000000	.000000
			12-23 Mo.	.000000	.000000	.000000
			2-65+ Yr.	.000000	.000000	.000000
Manganese Sulfate NAS 0124	28 Imit. Dairy(R)	*	0-5 Mo.	.000000	.000000	.000000
			6-11 Mo.	.077000	.126500	.077000
			12-23 Mo.	.044000	.187000	.044000
			2-65+ Yr.	.049500	.082500	.049500
Manganese Sulfate NAS 0124	83 Formulas (B)	4	0-5 Mo.	7.956090	14.575500	18.597780
			6-11 Mo.	1.621080	7.723830	3.789360
			12-23 Mo.	.521400	.146940	1.218800
Manganese Sulfate NAS 0124	All Categories	12	0-5 Mo.	8.920470	15.850300	19.577280
	**********		6-11 Mo.	9.129560	24.290400	11.472560
	**********		12-23 Mo.	15.898750	26.251220	16.748780
	**********		2-65+ Yr.	37.980350	57.079320	38.090950

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